(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 June 2004 (10.06.2004)

PCT

(10) International Publication Number
WO 2004/049095 A2

(51) International Patent Classification[7]: G06F

(21) International Application Number:
PCT/IB2002/004923

(22) International Filing Date:
25 November 2002 (25.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicants and
(72) Inventors: **MESSADEK, Jallal** [BE/BE]; Place des Béguinages 2, B-4000 Liège (BE). **ENNAMANY, Rachid** [MA/FR]; 13, rue Gérard Philippe, F-33140 Villenave d'Ornon (FR).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **THIRY, Michel** [BE/BE]; Cul du Bief 166, B-4870 Trooz (BE).

(74) Common Representative: **MESSADEK, Jallal**; Place des Béguinages 2, B-4000 Liège (BE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/049095 A2

(54) Title: BETAINE COMPOSITIONS

(57) Abstract: This invention relates to the pharmaceutical combination comprising at least: a first compound selected among the group consisting of acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of Formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound and in an amount by weight at least three times the amount of first compound.

BETAINE COMPOSITIONS

FIELD OF THE INVENTION

This invention relates to the pharmaceutical combination comprising at least:

- A first compound selected among the group consisting of acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and mixtures thereof, and

- A second compound selected from the group consisting of lipidic betaines, betaine lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound and in an amount by weight at least three times the amount of first compound.

Further, the invention relates to pharmaceutical composition comprising a betaine and aspirin in a formulation wherein the betaine and aspirin are formulated together in a bilayered tablet, the aspirin being present in a first layer, and the betaine being present in a second layer in an amount at least three times the amount of aspirin. Said pharmaceutical composition wherein the tablet includes a core and a coating layer surrounding said core and wherein one of the betaine and aspirin is present in the core and the other is present in the coating layer surrounding the core.

PRIOR ART

Glycine betaine, or betaine of formula $(CH_3)_3N^+ - (CH_2) - COO^-$, is a molecule known for its osmo-protective properties, its cosmetic and pharmaceutical uses.

WO 0051596 of one of the, the scope of which is incorporated by reference, discloses the use of betaine for the treatment of thrombosis not induced by homocystinuria. In examples, said application discloses the combination of glycine betaine with a contrast agent.

Our recent studies point out the activity of Betaines or/and compounds of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, preferably glycine betaine or a pharmaceutically acceptable salt thereof, esters thereof, precursors thereof, and mixtures thereof on P-selectin expression and to related diseases and pathologies induced by this glycoprotein. Consequently, therapeutic interventions directed against P-selectin or its ligand by compounds of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine n=1), or a pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, derivatives thereof and mixtures in the treatment thereof, may be beneficial in the treatment of thrombosis and in the treatment to related diseases and pathologies induced by this glycoprotein.

It is well know to administer to human doses of 1000 mg acetylsalicylic acid/day for treating pain relief, such as headaches, as well to administer doses of 100 mg to 500 mg acetylsalicylic acid /day as platelet anti aggregant for preventing thrombosis associated with atherosclerosis. These treatments are really effective, but have undesirable effects on patients subject to allergies or haemorrhage, especially when acetylsalicylic acid has to be administered every day, especially when acetylsalicylic acid has to be administered as platelet anti aggregant.

Despite its efficacy, antiaggregant treatment for preventing thrombosis with acetylsalicylic acid necessitates special precautions in use, such as overdose problems and unwanted side effects. This treatment makes it necessary to monitor patients, due in particular to haemorrhage-related problems which

can arise during or after medication, gastro-intestinal mucosa damages, as well as possible incompatibility with other drugs.

PCT/BE 02/ 00013 of one of the applicants, the scope of which is incorporated by reference, discloses a pharmaceutical combination comprising a therapeutic effective amount of a therapeutically active agent with at least one haemorrhagic side effect, and a therapeutic effective amount of a compound of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, preferably glycine betaine or a pharmaceutically acceptable salt thereof, esters thereof, precursors thereof, and mixtures thereof for preventing or reducing said haemorrhagic side effect and/or for potentialising the therapeutic effect of said active agent. As possible active agent with haemorrhagic side effect, acetylsalicylic acid is given as example. The dosage forms given is the example 33 of said document comprise:

- acetylsalicylic acid 500 mg + 500 mg betaine + excipient
- acetylsalicylic acid 300 mg + 200 mg betaine + excipient, and
- acetylsalicylic acid 300 mg + 400 mg betaine + excipient.

US Patent 4,703,045 discloses a therapeutic composition containing betaine salts for the treatment of hangover, said oral composition comprising a pain relieving amount of analgesic (see claim 1 of said patent). In the example, unit doses according to compositions 4 and 12 of said patent comprises 200 mg acetylsalicylic acid for 2000 mg betaine citrate and other excipient, while unit dose of composition 15 comprises 110 mg acetylsalicylic acid for 2000 mg betaine and other excipient. The effervescent tablet of composition 19 comprises 250 mg of acetylsalicylic acid for 1750 mg betaine citrate and other excipient. Unit composition containing less than 100 mg acetylsalicylic acid does not contain a pain relief amount of analgesic for a human having a weight of about 70 kg.

US Patent 6,235,311discloses a pharmaceutical composition which is useful for cholesterol lowering and reducing the risk of a myocardial infarction, which includes a statin, such as pravastatin, lovastatin, simvastatin, atorvastatin, cerivastatin or fluvastatin, in combination with aspirin, in a manner to minimize interaction of aspirin with the statin and minimize side effects of aspirin. This pharmaceutical composition comprising a statin cholesterol lowering agent and aspirin in a formulation to reduce statin/aspirin interaction wherein the statin and aspirin are formulated together in a bilayered tablet, the aspirin being present in a first layer, and the statin being present in a second layer. The pharmaceutical composition as claimed in said patent is a the tablet including a core and a coating layer surrounding said core and wherein one of the statin and aspirin is present in the core and the other is present in the coating layer surrounding the core.

French Pat. No. 2590 M of 1963 describes the combination of betaine citrate with aspirin to buffer aspirin were the highest ratio of betaine citrate/aspirin is 5:3 i.e. 1,67 times the amount by weight of betaine citrate versus aspirin. The lowest amount of aspirin in each unitary dose is 300 mg.

French Pat. No. 1123 M of 1962 describes the synthesis of a betaine salicylate. Betaine salicylate is prepared through the interaction of betaine base and salicylic acid in anhydrous alcoholic medium with a ratio of 1.2: 1, i.e. 1,2 times the amount by weight of betaine versus aspirin

None of these publications describe a pharmaceutical combination, advantageously as an unitary dose, wherein acetylsalicylic acid is less than 80 mg and Betaines is at least three .times by weight the amount of acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof and mixtures thereof.

None of these publications describe a pharmaceutical composition comprising a betaine and aspirin in a formulation wherein the betaine and aspirin are formulated together preferably in a bilayered tablet, the aspirin

being present in a first layer, and the betaine being present in a second layer in an amount at least three times by weight the amount of aspirin.

None of these publications describe a pharmaceutical composition wherein the tablet includes a core and a coating layer surrounding said core and wherein one of the betaine and aspirin is present in the core and the other is present in the coating layer surrounding the core.

None of these publications describe a pharmaceutical composition wherein the tablet includes a core and a coating layer surrounding said core and wherein one of the betaine and aspirin is present in the core and the other is present in the coating layer surrounding the core and one or more of the Betaines and aspirin are formulated as a controlled release formulation.

None of these publications describe a pharmaceutical composition wherein the tablet includes a core constituted by aspirin and/or its pharmaceutically acceptable derivatives and a coating layer surrounding said core wherein one or more of the Betaines is present and formulated as a controlled release formulation.

None of these publications describe the therapeutically synergistic effect of betaine and aspirin allowing aspirin unitary dose amount reduction to less than 80 mg, while improving its therapeutic effect.

It has now been observed that it was possible to reduce the daily dose of acetylsalicylic acid for a human with a weight of 70 kg to less than 100 mg, when administrated in combination with a betaine while preventing thrombosis without special precautions in use, without overdose problems and without unwanted side effects. This treatment makes it possible to no more render necessary to monitor patients for haemorrhage-related problems which can arise during or after medication, as well as possible incompatibility with other drugs. Moreover, betaine prevents acetylsalicylic acid gastro-

intestinal induced mucosa damage when administrated in combination. Due to betaine antithrombotic properties it have appears that administrating the pharmaceutical combination of the invention allows to substantively reduce the amount of acetylsalicylic acid while achieving a significant therapeutic effect. In fact betaine and acetylsalicylic acid act in a synergistic manner with a good therapeutic effect in various pathologies such as blood flow disturbances, thrombo-embolism, inflammation and cancer.

The invention relates thus among others to:

Pharmaceutical combination as a unitary dose comprising at least:

- A first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and or salts thereof, and mixtures thereof, and
- A second compound selected from the group consisting of betaine lipids, lipidic betaines, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, or pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound

in which said combination comprises less than 80 mg of said first compound expressed as acetylsalicylic acid, and

in which the amount of second compound is at least three times the amount, calculated as acetylsalicylic acid, of said first compound.

BRIEF DESCRIPTION OF THE INVENTION

The use of aspirin for reducing the risk of a myocardial infarction and the use of betaine for preventing or treating atherosclerosis and cardiovascular disease and cerebrovascular disease are well documented.

Aspirin is known for causing gastrointestinal bleeding when used for long-term therapy. It is therefore desirable in long-term aspirin therapy that the aspirin is provided in a form and an amount which minimizes side effects.

The aim of the present invention is to lower the aspirin amount needed to achieve a therapeutically effect when combining aspirin with betaine. Due to betaine antithrombotic properties the two drugs act in a synergistic manner for a powerful effect. Moreover betaine reduces aspirin induced side effects as haemorrhage and gastrointestinal damages. The combination of the invention may be useful for long term therapies as vascular occlusive diseases, inflammation, cancer and diabetes and aging related pathologies.

In view of the above, it is seen that there is a long-felt want in patients required to take both a betaine and aspirin for a betaine-aspirin formulation which provides for maximum reduction of risk of a myocardial infarction without the undesirable side effects and drug interaction normally associated with use of such combination.

Glycine betaine, as well as betaine compounds of the general formula $(CH_3)_3N^+ - (CH_2)_n - COO^-$, with n varying from 1 to 5 (preferably equal to 1) in the context of the present invention can be used in combination with acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof for various clinical applications, such as: coronary thromboses and venous thromboses

- thrombosis and re occlusion of the vascular system following a thrombolysis or an angioplasty
- ischemia reperfusion
- acute disseminated intravascular coagulation
- P-selectin related troubles
- infarct, angina pectoris, aneurysm, pulmonary embolism, phlebitis
- cerebral, thrombosis and thrombo-embolism
- post-traumatic shock, whether or not of surgical origin

- prevention of accidents of microcirculation in the following cases: haemophilia, chemotherapy, ageing, oral contraception using oestrogen's, obesity, tobacco addiction, prosthesis, claudication, diabetes.
- prevention of the risks associated with the administration of contrasting ionic and non ionic products.
- The extracorporeal circulation and the haemodialysis procedures. The blood in contact with artificial surfaces of patients subject to an extracorporal circulation has a risk of formation of platelet nails, of thrombi and embolism. These risks can be prevented by administering the compound(s) of the invention before and/or during and/or after these events.
- Reduction of atherosclerotic events (myocardial infarction, stroke, death due to vascular causes and restinosis) in patients with a history of symptomatic atherosclerotic disease defined by ischemic stroke, myocardial infarction or established peripheral arterial or venous disease.
- Inflammation phenomena. When binding it with integrine of Mac-1 receptor of the leukocytes and by reducing the expression of mitogenes and pro inflammation cytokines. When acting on the Mac-1 receptor, the compounds of the invention reduce the adhesive and migration properties of the leukocytes reducing thereby the tissue aggression.
- Stings and bites of venomous animals. Experimental data show that the injection of compounds of the invention to rats to which a venom lethal dose is injected, delays the death thereof. The compounds of the invention are therefore suitable for entering into antidote composition for venom, possibly in combination with other antivenomous compound(s).
- Prevention of blood circulation problems due to contact with artificial surfaces, such as biomaterial elements, prosthesis, etc. (balloons, catheters, hip prosthesis, stents, prosthetic cardiac valves, arterial gafts, etc.). When using these elements with the compounds of the invention, the secondary effects are reduced. Moreover coating these exougenous

materials with the compounds of the invention avoid problems as reocclusion, rethrombosis and restinosis.

- Metastasis Prevention of cancerous cells. This anti tumoral activity is bound to the fact that cancerous cells released from tumours are transported by the micro thrombi inside the vascular system. These cancerous cells are undetectable by the immune system able to destroy them. Moreover, their incorporation in the micro thrombi facilitates their binding to the vascular system or in the organs, and creates then new cancerous colonies. As the formation of thrombi is function to the adhesion of fibrinogen to glycoprotein IIb IIIa site on the activated platelets, an antagonist of fibrinogen adhesion has an anti-metastasis activity by permitting the immune system to detect the cancerous cells during their migration, and by removing the vehicle (thrombus) enabling their transport and their binding. The compounds of the invention can be administered alone or with other anti-cancerous compounds (simultaneous administration or not) so as to improve their efficiency and the process of angiogenesis during malignant melanomas.
- Process for avoiding thrombo-embolic problems correlated to air trips. In view of its very low toxicity and its blood fluidifying characteristics, the compounds of the invention can be administered in the form of patch, sweets, confectioneries, cookies, drinks, meals, candies, etc. so as to prevent thromboembolic events for airplane /flight passengers.
- Sweetener for diabetes, the betaine being or not associated with another sweetener. As Betaine is a residue of sugar production, betaine has some sweetening properties which can be used for the preparation of sweetener with anti aggregation properties. Said sweetener, while avoiding circulation problems bound to diabetes, could improve the efficiency of insulins. It has been demonstrated that the activation of vitronectin receptors facilitates the cell migration and provides the necessary signals for the regulation and proliferation of cells, and potentialises the insulin effect (Ruoslahti, Kidney Int., 1997, 51, 1413-1417)

- As anti bacterial and anti infectious
- In combination with antibiotics
- In combination with insulin
- In combination with non steroidal anti inflammatory drugs
- Use of a compound of the invention for the treatment or for the prevention of troubles bound to one or more glycoprotein, especially to the receptor of one or more glycoprotein, preferably to the receptors of glycoprotein Ib and IIb IIIa.
- Use of a compound of the invention for potentializing the therapeutically effect of a pharmaceutical active agent.

DESCRIPTION

The pharmaceutical compositions of the invention which includes a combination of a betaine and aspirin is effective in preventing, reducing and/or treating atherosclerosis, cardiovascular events and disease including coronary events and cerebrovascular events, and coronary artery disease and/or cerebrovascular disease, cancer, inflammation, diabetes and troubles related to aging.

The terms "cardiovascular event(s)" and "cardiovascular disease" as employed herein refer to coronary and/or cerebrovascular event(s) and disease including primary myocardial infarction, secondary myocardial infarction, myocardial ischemia, angina pectoris (including unstable angina), congestive heart failure, sudden cardiac death, cerebral infarction, cerebral thrombosis, cerebral ischemia, transient ischemic attack and the like.

The term "coronary artery disease" (CAD) as employed herein refers to

diseases including atherosclerosis of the coronary arteries, previous myocardial infarction, ischemia, angina pectoris and/or heart failure.

The term "cerebrovascular disease" as employed herein refers to diseases including atherosclerosis of the intracranial and/or extracranial arteries, cerebral infarction, cerebral thrombosis, cerebral ischemia, stroke, and/or transient ischemic attacks.

The term" Betaines" as employed herein refers to compounds selected from the group consisting of lipidic betaines, betaine lipids, and/or betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, (preferably glycine betaine n = 1), pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof.

The terms "lipidic betaines" and "betaine lipids" refer to betaine lipids which are structural components of membranes commonly found in ferns, mosses, fungi, amoeba, eukaryotes such as nonseed plants and algae. Betaine lipids are ether-linked, nonphosphorous glycerolipids that resemble the more commonly known phosphatidylcholine in overall structure. Most common glycerolipids are containing a diacyl-glycerol moiety to which a polar head group is attached. This head group can be a carbohydrate moiety as in the very abundant plant galactolipids or a phosphorylester as in the glycerophospholipids, the most common lipid class in animals. Betaine lipids represent a third class of glycerolipids in which a quaternary amine alcohol is bound in an ether linkage to the diacylglycerol moiety. They can be obtained by extraction, by biosynthesis or by synthesis. The betaine lipid diacylglyceryl- *O*-4' – (N, N, N- trimethyl)homoserine and a closely related isoform diacylglyceryl - *O*-2' – (hydroxymethyl) (N, N, N- trimethyl)-β-alanine are the most common.

Aspirin will preferably be employed in the form of salicylic acid acetate also referred to as acetylsalicylic acid.

In one embodiment salicylic acid may be employed.

In one embodiment betaine salicylate as described in French Patent 1.123 M of 1962 may be employed.

In one embodiment mixtures of one or more salicylic acid, acetylsalicylic acid and betaine salicylate may be employed.

Background

Platelet aggregation is an essential event in the formation of blood clot and thrombus. In normal conditions, following a vascular lesion, blood clots prevent blood losses by closing the opening. However, in some pathological instances, the formation of a blood clot can reduce partly or completely the blood circulation, with the consequence of a cellular necrosis.

For example, the platelet aggregation and thus the thrombosis at the level of the atherosclerosis plaques is an important factor for the genesis of conditions such as angina pectoris, myocardium infarct and vessel occlusion following a thrombolysis or an angioplasty. Patients suffering a heart attack are treated with thrombolytic agents such as plasmin activators and the streptokinases which dissolve the fibrin from the clots. A major complication of this therapy is the reocclusion of vessels due to platelet aggregation, which can lead to irreversible damages to the heart, the brain or other organs.

Thrombosis starts with the adhesion of platelets at the vascular lesion sites. The platelet adhesion is initiated by the receptor located at the surface of the platelets which bind to proteins of the extracellular cellular matrix of the exposed endothelium, such as fibrinogen, fibronectin, Von Willebrand factor, as well as other adhesive proteins such as vibronectin, collagen and laminin. Therefor, the activation of platelets is a reply to agonists such as epinephrine, ADP, collagen, the arachidonic acid or the thrombin. This activation leads to

the activation of the glycoprotein Ib receptor (GP Ib) and/or of the glycoprotein IIb IIIa receptor (GP IIb IIIa) at the surface of the platelets. This receptor(s) (GP Ib and/or GP IIb IIIa) is/are then available for its/their binding to fibrinogen and the platelet aggregation. The adhesion of the receptor (GP IIb IIIa) to other adhesive proteins such as the Von Willebrand factor also leads the attachment of platelets between them and their aggregation. The adhesion of molecules such as fibrinogen or the Von Willebrand factor to the receptor (GP IIb IIIa) leading the platelet aggregation is an essential step in the formation of the thrombus. The receptor (GP IIb IIIa) is thus a privileged target for the new therapy treating thrombosis and thromboembolic pathologies. Furthermore, the use of antagonists of the glycoprotein IIb IIIa receptor inhibits the platelet aggregation, while respecting the other haemostasis mechanisms, is highly desirable in the new therapies bound to thrombosis. Several molecules having this antagonist property are marketed with usage restrictions due to immuno-reactivity problems, toxicity, allergy or hypersensibility reactions for some patients. A subject matter of the present invention is to propose a molecule, especially a well-known and used molecule of vegetal origin, having this antagonist activity for the glycoprotein IIb IIIa receptor, while not having toxic characteristics.

It is also known that the activation of the vitronectin receptor improves the cell migration and provides regulating signals of the cell proliferation and cell differentiation, and activates the effects of insulin (Ruoslahti, Kidney Int., 1997, 51, 1413-1417). The regulation of the vitronectin receptor is associated with pathological conditions, such as vascular restinosis (Clemetson and Clemetson, Cell.Mol. Life Sci., 1998,54,502-513), bone excess resorbtion (Rodan and Rodan, J. Endocrinol., 1997, 154 Suppl, S47-56), and the angiogenesis process during the malignant melanomas (Cheresh, Cancer Metastasis Rev., 1991, 10,3-10).

Surprisingly, it has now been found that betaines of formula $(CH_3)_3N^+ - (CH_2)_n - COO^-$, with n an integer from 1 to 5, and their pharmaceutically acceptable salts, have an antagonist activity for one or more glycoprotein(s) receptors, such as the glycoprotein Ib receptor and the glycoprotein IIb IIIa receptor, by inhibiting the platelet aggregation induced by various agonists. This antagonist activity is not restricted to the glycoprotein site IIb IIIa but to all glycoprotein sites implicated in the cell adhesion of various origins, there between.

Surprisingly, it has now been found that betaines of formula $(CH_3)_3N^+ - (CH_2)_n - COO^-$, with n an integer from 1 to 5, and their pharmaceutically acceptable salts when combined to acetylsalicylic acid act in a synergistic manner in different pathologies. This synergistic activity permit to lower the aspirin amount needed to achieve a therapeutically effect.

Platelets are activated by some agonists, whereby their forms, as well as their secretions of their granules can be modified, and whereby the aggregation thereof can be induced and the formation of clots and thrombi can be produced.

The present used platelet aggregation inhibitors are acting only on a single agonist. For example, aspirin is active against the arachidonic acid, ticlopidin is active against ADP, hirudin is active against thrombin. The Betaines of the general formula of the invention disclosed here before are actives against various agonists, as well as on fibrinogen, fibronectin, Von Willebrand factor and other adhesive proteins such as P-selectin, vitronectin, collagen, laminin families and lectin families. This is a major improvement for their efficiency, while preserving the haemostasis mechanism so as to avoid haemorrahgic or bleeding events. Due to their activity by oral administration, said compounds are excellent candidates for pathologies with adhesion of cells between them.

In view of its very low toxicity and its efficiency, the best results have been obtained with glycine betaine (compound of the general formula with n = 1).

None of the publications to which reference is made in the present specification teach the antagonist activity of the betaine/aspirin combination with respect to glycoprotein IIb IIIa receptor, nor its activity with respect to adhesive proteins. This antagonist activity is not only limited to the site of glycoprotein IIb IIIa, but also to all the other glycoproteic sites acting in the adhesion of cells of various origins there between.

In the present invention, pharmaceutically acceptable salts are salts of betaine which can be administered, such as salts of betaine with hydrochloric acid, sulfuric acid, sulfonic acid, organic acids such as acetic acid, citric acid, tartaric acid, formic acid, salicylic acid, acetylsalicylic acid, etc., as well as the monohydrate radical.

Acetylsalicylic acid, salicylic acid and pharmaceutically acceptable salts are known to have platelet antiaggregant properties. However, the daily dose for ensuring a safe anti-aggregation for human with a weight of 70kg is at least 75 to 500 mg acetylsalicylic acid/ day. High daily dose causes high bleeding risks, whereby requiring monitoring patients, due in particular to haemorrhage-related problems which can arise during or after medication. With respect to low doses, it is also required to monitor the patients so as to check that the low doses are effective for ensuring the required platelet anti-aggregation. It is not possible for the moment to determine before the treatment is made whether the low dose of aspirin will be sufficient for a patient. For theses reasons, patients requiring an administration of aspirin as platelet antiaggregant receive daily doses of at least 125 mg.

It has now been discovered that it was possible to reduce the daily doses of aspirin and/or the requirement of monitoring the patient, while ensuring the prevention of arterial and venous thrombosis for substantially all types of patients in need of treatment and while avoiding substantially all bleeding risks, haemorrhage-related problems and aspirin induced gastro-intestinal

damages which can arise during or after medication, as well as possible incompatibility with other drugs.

The invention relates thus to a pharmaceutical combination as a unitary dose comprising at least:

- A first compound selected among the group consisting acetylsalicylic acid, salicylic acid, mixtures thereof, pharmaceutical derivatives thereof, and
- A second compound selected from the group consisting of betaine lipids, lipidic betaines, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine), pharmaceutically acceptable salts thereof, pharmaceutically acceptable esters thereof, pharmaceutically acceptable precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound

in which said combination comprises less than 100 mg of said first compound expressed as acetylsalicylic acid, and

in which the amount of second compound is at least three times the amount, calculated as acetylsalicylic acid, of said first compound.

The pharmaceutical combination is for example suitable for a four times a day administration or preferably for a daily administration. (advantageously a two times a day administration, preferably an once day administration).

Oral formulations

Advantageously, the combination comprises an amount of said first compound, calculated as acetylsalicylic acid, of less than 80 mg, advantageously of less than 60 mg, preferably of less than 40 mg, even if said combination is administered as a daily administration in one or more doses.

For example, the combination comprises an amount of acetylsalicylic acid or pharmaceutical derivative thereof corresponding to 5 to 80 mg, advantageously from 10 to 75 mg, preferably from 15 to 75 mg calculated as acetylsalicylic acid. Specific examples of combination comprise 10, 15, 20, 25,30,40,50 and 70 mg of acetylsalicylic acid.

Advantageously, the amount of second compound is at least 5, advantageously at least 10 times the amount, calculated as acetylsalicylic acid, of said first compound, preferably at least 20 times the amount, calculated as acetylsalicylic acid, of said first compound. For example, the amount of second compound in the combination is 30, 40, 50, 70, 85, 100 times the amount, calculated as acetylsalicylic acid, of said first compound.

According to a specific embodiment, the combination is prepared at least from a mixture in which at least 50% by weight of the first compound and at least 50% of the second compound are in soluble form. Preferably, the combination is prepared at least from a mixture in which at least 90% by weight of the first compound and at least 90% of the second compound are in soluble form, most preferably from a mixture in which the first compound and the second compound are substantially completely in soluble form. This is advantageous for ensuring a homogeneous dispersion of the active compounds in the batch used for the preparation of the unit dose.

The combination of the invention is advantageously at least a controlled release combination for the second compound and/or at least an immediate release combination for the first compound. Preferably, the combination is a controlled release formulation for at least a part of the second compound and a substantially immediate release formulation for the first compound. The combination is for example a formulation enabling an immediate release for the first compound and for an amount of the second compound corresponding to two to ten times the amount of the first compound, and enabling a

controlled release for an amount of second compound corresponding to more than 2 (advantageously more than 5, preferably more than 10, most preferably more than 20) times the amount of first compound. The weight ratio amount of second compound in a controlled release form / amount of second compound in an immediate release form is for example comprised between 2:1 and 100:1, advantageously between 5:1 and 75:1, preferably between 10:1 and 50:1.

Possibly, the first compound can also be partly in a form suitable for a controlled release.

According to a specific embodiment, the combination is a formulation ensuring a first immediate release of an amount of second compound, then an immediate release of the first compound, and thereafter a controlled release of an larger amount of second compound.

The weight ratio amount of second compound in a controlled release form / amount of second compound in an immediate release form is for example comprised between 2:1 and 100:1, advantageously between 5:1 and 75:1, preferably between 10:1 and 50:1.

Immediate release form means in the present specification a form from which an active compound is released in the body so as to be bioavailable, less than 30 minutes after administration, advantageously less than 15 minutes after administration.

Controlled release form means in the present specification a form from which the release of an active compound is controlled during the time, such as delayed release and/or extended release. Advantageously, the controlled release form is a form suitable for ensuring a minimum active agent concentration in the blood during at least 4 hours, advantageously during at least 6 hours, preferably during at least 8 hours, most preferably during 12 hours or more than 12 hours , such as during 24 hours or more.

According to an embodiment, the combination comprises dry particles, especially micro particles, prepared by drying a mixture in which the first compound and the second compound are partly in a soluble form. In such dry particles, the first compound is homogeneously dispersed in the second compound, whereby enabling a simultaneously release of the first compound and of the second compound.

The first compound and the second compound can also be combined in the form of a matrix and or in the form of a mixture of dry particles and/or in the form of a suspension, solution, etc.

When the first compound and the second compound are combined in the form of a solution, the solution can be absorbed in porous carrier, such as porous matrix, porous solid particles, etc., the porous carrier containing the solution of first and second compounds can then be coated with a layer, such as an enterosoluble film layer, a gastric soluble layer, a controlled release layer, a layer for ensuring an extended release or a delayed release. The porous carrier containing the solution of first compound and second compound can be submitted to treatment, such as heat treatment for ensuring a deposit of first compound and/or second compound in the pores or on surface of the pores and/or for increasing the viscosity of the solution in the pores. The advantage of using such porous carrier containing the first and second compound in soluble form or in a substantially soluble form is that the release of first compound and second compound from the porous carrier occurs in the form of a solution, whereby facilitating the bioavailability of the active agent.

The porous carrier has for example a mean particle size of less than 5000 μm, such as less than 2000μm, advantageously less than 1000μm, for example a size comprised between 100μm and 800μm, such as an average particle size of 200μm, 300μm, 400μm, 500μm,600μm, 700μm and 750μm. The average pore diameter or average pore opening size is sufficient for enabling an easy passage of the solution into the pores, such diameter or size being for

example lower than 20μm, such as lower than 10μm, advantageously lower than 2μm, preferably in average (average in number or average in volume) lower than 1μm, such as comprised between 5nanometer and 750nanometer, for example between 20nanometer and 600nanometer. The average size in volume can be estimated as being equal to (4 x total volume of the pores or porosity)/ (surface or specific surface). According to a specific embodiment, the average size in volume is determined by taking into account the pore volume formed by pore with a diameter or size greater than 2 nanometers, advantageously greater than 5 nanometers, and the specific surface or BET surface of the pores with a diameter or size greater than 2 nanometers, advantageously greater than 5 nanometers.

According to a still further possible embodiment, the combination further comprises at least one compound reacting in presence of water so as to prepare a substantially immediate solution of first compound and second compound. For example, the combination is an effervescent combination enabling the preparation of an aqueous solution containing the first and second compounds, in a very short time, substantially immediately, advantageously substantially without mechanical shaking.

The invention relates also to a pharmaceutical unit dose comprising at least a pharmaceutical combination containing at least:

- A first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, mixtures thereof, and
- A second compound selected from the group consisting of lipidic betaines, betaine lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine), pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound ,

In which the combination is prepared from a mixture in which the first compound and the second compound are partly in a soluble form,
Whereby the pharmaceutical unit dose comprises less than 100 mg, advantageously less than 80 mg (such as less than 75 mg, preferably less than 50mg) of said first compound expressed as acetylsalicylic acid, and
in which the amount of second compound is at least three times the amount, calculated as acetylsalicylic acid, of said first compound.

The combination of said unit dose is advantageously prepared from a mixture in which at least 50% by weight of the first compound and at least 50% of the second compound are in soluble form, preferably from a mixture in which at least 90% by weight of the first compound and at least 90% of the second compound are in soluble form, most preferably from a mixture in which the first compound and the second compound are substantially completely in soluble form.

The combination is for example in the form of dry particles, especially micro particles, prepared by drying a mixture in which the first compound and the second compound are partly in a soluble form.

A combination as an oral unit dose, wherein the first compound is in a form of a core in the form of dry particles, especially micro particles prepared by drying acetylsalicylic acid, salicylic acid, pharmaceutical derivatives and mixtures thereof, and a second compound partly or completely in a soluble form selected from the group consisting of lipidic betaines, betaine lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, said second compound in a amount at least three times by weight the amount of the first compound and partially or completely making a coating for the first compound.

The combination of the pharmaceutical unit dose of the invention can have one or more characteristics of the combination of the invention as disclosed here above.

The invention further relates to a kit for a daily administration, said kit comprising at least:

- An first oral formulation comprising at least a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and
- A second oral formulation comprising at least a second compound selected from the group consisting of lipidic betaines, betaine lipids, betaine of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine), pharmaceutically acceptable salts hereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound

in which the first oral formulation comprises less than 250, advantageously less than 100 mg of said first compound expressed as acetylsalicylic acid, and in which the amount of second compound in the second oral formulation is at least three times the amount, calculated as acetylsalicylic acid, of said first compound.

Advantageously, the first oral formulation comprises an amount of said first compound, calculated as acetylsalicylic acid, of less than 85 mg, advantageously of less than 75 mg, preferably of less than 60 mg. For example, the first oral formulation comprises an amount of acetylsalicylic acid or pharmaceutical derivative thereof corresponding to 5 to 80 mg, advantageously from 10 to 75 mg, preferably from 15 to 75 mg calculated as acetylsalicylic acid.

According to a specific embodiment, the second oral formulation comprises an amount of second compound corresponding to at least 10 times the amount, calculated as acetylsalicylic acid, of said first compound.

The kit is for example two distinct oral formulations to be administered successively immediately or to be administered at different moment of the day. For example, in the morning, the first oral formulation is administered, while at midday and/or in the afternoon and/or in the evening a second oral formulation is administered. The kit can thus comprise more than two oral formulations to be administered during a day. The first oral formulation can contain an amount of a second compound (different from the first compound), while the second oral formulation can contain an amount of a first compound (different from the second compound). According to a specific embodiment, the first oral formulation and the second oral formulation are substantially identical or similar. However, preferably the second oral formulation has a higher content of second compound and is poor in first compound. The kit can comprise more than 2 oral formulations, for example three, four, etc oral formulations for administering a one day dose. The oral formulations of a kit can be administered in different forms. For example a first oral administration to be taken as a dry formulation (tablet, pills, etc), while the second administration has to be taken as a syrup, solutions, etc.

Advantageously, the second oral formulation and/or third oral formulation comprise an amount of second compound corresponding to at least 20 times the amount, calculated as acetylsalicylic acid, of said first compound.

The first oral formulation comprises also an amount of a second compound selected from the group consisting of lipidic betaines, betaine lipids, betainesof formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine), pharmaceutically acceptable salts thereof, esters

thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound.

For example, the first oral formulation is prepared at least from a mixture in which at least 50% by weight of the first compound and at least 50% of the second compound are in soluble form, advantageously at least from a mixture in which at least 90% by weight of the first compound and at least 90% of the second compound are in soluble form, preferably at least from a mixture in which the first compound and the second compound are substantially completely in soluble form.

Advantageously, the second oral formulation is at least a controlled release formulation for the second compound.

Preferably, the first oral formulation is at least an immediate release formulation for the first compound and possibly for an amount of the second compound.

According to a specific form of a kit of the invention requiring two oral administrations per day, the first administration is an oral administration of an oral formulation which is substantially an immediate release formulation for the first compound and for an amount of second compound different from the first compound (for example comprised between 0.5 and 10 times the amount of first compound, advantageously comprised between 1 and 5 times the amount of the first amount) and which is advantageously a controlled release for an amount of the second compound (for example release controlled for more than 8 hours, such as release controlled for 10 hours, 12 hours or even more), while the second oral formulation (for example to be taken 12 hours after the administration of the first oral formulation) is at least a controlled release formulation for the second compound (for example release controlled for more than 8 hours, such as release controlled for 12 hours, 24 hours or

even more), said second oral formulation being preferably substantially free of first compound.

The use of

- A first compound selected among the group consisting of acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, mixtures thereof, and
- A second compound selected from the group consisting of lipidic betaines, betaine lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine), pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound ,

for the preparation of a pharmaceutical combination for treating or preventing blood flow disturbances, and/or
for the preparation of a pharmaceutical combination for treating or preventing inflammation, and/or
for the preparation of a pharmaceutical combination for treating or preventing cancer, and/or
for the preparation of a pharmaceutical combination for treating or preventing diabetes and/or
for the preparation of a pharmaceutical combination for treating or preventing vascular diseases and/or
for the preparation of a pharmaceutical combination for treating or preventing at least one trouble related to aging and/or

The invention relates also to a pharmaceutical kit comprising at least one pharmaceutical combination of the invention or one pharmaceutical unit dose according to the invention , and a second pharmaceutical unit dose containing

as active agent at least a compound selected from the group consisting of lipidic betaines, betaine lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5 (preferably glycine betaine), pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second unit dose is free of compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof.

The invention further relates to a process of treatment of a patient in need for treating or for preventing thrombosis troubles for a patient, by administering to said patient a pharmaceutical combination according to the invention or a pharmaceutical unit dose according the invention, in which advantageously before and/or during and/or after said administration, a therapeutic effective amount of glycine betaine is administered to said patient for preventing or reducing the haemorrhagic side effect.

Acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof and Betaines, preferably glycine betaine, are advantageously administered orally, parenterally, sub cutaneously, by suppositories, tablets, capsules, syrup, etc. Administered doses can vary from 0.001g to 1 g per kg live body, for example from 0.005 g to 0,5 g, in particular from 0.01 g to 0,3 g per kg life body.

Examples of administration forms are: tablets, capsules, patches, injectable forms, releasing forms, sublingual administration form, powder (for example for inhalation therapy, buccal inhalation), syrup, solution (nebulization, for example for inhalation therapy, buccal inhalation). As preferred administration forms, subcutaneous injectable dosage form, patches (to be applied on the skin) and entero soluble oral dosage form, such as gastro

insoluble tablets or capsules, etc. provided with an entero soluble coating or matrix or system.

The pharmaceutical combination can be in the form of a kit, so as to prepare the combination before administration or during the administration.

It is an object of the present invention to provide an oral, rectal, parenteral, transdermal, extracorporal, intracorporal controlled release of a betaine, preferably glycine betaine preparation suitable for at least 5 minutes, such as for at least 10 minutes, ...twelve-hourly (e.g. up to twenty-four hourly or even more, such as for a week, two weeks, one month, three months) in combination with acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof for the treatment of a mammalian.

In accordance with the present invention, a pharmaceutical composition is provided which includes a betaine and aspirin, which provides for maximum patient benefits including maximum reduced risk of a myocardial infarction with minimal physical and chemical incompatibility (including minimal betaine/aspirin interaction), and reduced side effects normally associated with use of aspirin.

In addition, in accordance with the present invention, a method is provided for preventing or inhibiting or treating atherosclerosis, and/or reducing risk of or treating a cardiovascular event or disease including coronary artery disease and cerebrovascular disease, wherein a pharmaceutical composition containing a combination of a betaine agent and aspirin in a single dosage form, in a manner so as to minimize interaction of the betaine and aspirin, is administered to a patient in need of treatment.

Preferred pharmaceutical compositions of the present invention may take the

form of several different embodiments. Thus, in one embodiment of the present invention, a pharmaceutical composition is provided wherein the betaine (including any betaine agent) and aspirin are formulated together in a single tablet. The tablet of the invention is preferably in the form of a bilayered tablet which includes a first layer and a second layer. Aspirin, in the form of granules of preselected size will be present in the first layer together with optional excipients as described hereinafter, while the betaine will be present in the second layer which optionally may include one or more buffering agents (as necessary to prevent undesirable betaine/aspirin interaction) and optionally one or more excipients as described hereinafter. The betaine will be present in the second layer optionally in a slow and/or controlled release form.

In addition, the bilayered tablet of the invention may include an outer protective coating or finishing layer as described hereinafter.

In addition, the bilayered tablet of the invention may include further another therapeutically active agent.

In addition, the bilayered tablet of the invention may include further another therapeutically active agent as listed in the application PCT/BE 02/ 00013 of the one of the applicants.

Another embodiment of the present invention comprises a cored tablet which includes a core and a buffering layer or outer coat which can be compressed onto the core as a dry coat. The core will preferably include compressed aspirin granules while the buffering layer or outer coat will include a betaine (including any betaine agent) together with one or more buffering agents and optional excipients.

Provision of aspirin in the core and betaine in the buffering layer will

effectively reduce the aspirin dose needed, reduce its side effects and also minimize drug incompatibilities while providing maximum efficacy.

The so-described cored tablet may also optionally include an outer protective coating or finishing layer as described hereinafter.

In addition, in accordance with the present invention, a pharmaceutical composition is provided which is in the form of a tablet or capsule which includes a mixture of aspirin granules having an enteric coating and particles or granules of a betaine. Such a combination will provide maximum efficacy while minimizing side effects resulting from prolonged aspirin therapy.

In the above embodiment containing enteric coated aspirin, the betaine may include any betaine agent, but preferably glycine betaine.

In the above embodiment containing enteric coated aspirin, the betaine may include any betaine agent, but preferably lipidic betaine and/or betaine lipids.

In yet another embodiment of the pharmaceutical composition of the present invention, enteric coated aspirin granules as described above may be further coated with a protective coating or finishing layer. The double coated particles of aspirin can be mixed with any Betaines such as compounds of general formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, preferably glycine betaine or a pharmaceutically acceptable salt thereof, esters thereof, precursors thereof, and mixtures thereof, powders or granules, and the mixture can be encapsulated or tableted as described herein. This combination will protect the integrity of the enteric coat and minimize the side effects normally resulting from prolonged aspirin therapy. The aspirin and the betaine granules do not need to be mixed together; these can even be encapsulated separately into the same capsule shells in two shots.

In the above embodiment containing enteric coated aspirin, the betaine may include any Betaines agent, but preferably glycine betaine monohydrate.

In the above embodiment containing enteric coated aspirin, the betaine may include any Betaines agent, but preferably lipidic betaine and/or betaine lipids.

Another embodiment of the pharmaceutical composition of the invention includes granules of enteric coated aspirin and enteric coated betaine (including any Betaines agent), in the same dosage form such as compressed tablets or capsules.

The tablets containing the enteric coated granules of aspirin and betaine may also include an outer protective coating or finishing layer.

In a further embodiment of the pharmaceutical composition of the invention, where aspirin side effects are not an issue, for example, where low dose aspirin is present (80 mg or less), the composition of the invention may comprise a mixture of aspirin granules and betaine (including any Betaines agent, preferably, glycine betaine or enteric coated particles of betaine or particles of betaine, containing an outer protective coating or finishing layer); the above mixture may take the form of compressed tablets or capsules (where the mixture can be encapsulated separately in two shots in the same capsule shells).

The pharmaceutical composition of the invention in the form of a tablet or capsule will include aspirin in amounts from about 5 to about 800 mg, preferably 10 to 350 mg, most preferably less than 100mg.

The aspirin for use in forming the pharmaceutical composition of the invention will preferably be in the form of granules having an average particle size within the range from about 10 μm to about 2 mm, more preferably from about 0.25 mm to 1.0 mm.

The pharmaceutical composition of the invention will contain a Betaine such as glycine betaine, lipidic betaines and/or betaine lipids, in an amount as

normally employed for such betaine as exemplified in the patents thus, depending upon the particular betaine, it may be employed in amounts within the range from about 0.1 mg to 20000 mg per day in single or divided doses, and preferably from about 0.2 to about 10000 mg per day. Most preferably for betaine, a daily dosage in single or divided doses of 100 to 5000 mg, such as 300mg, 500mg, 750mg, 1000mg, 1500mg, 2000mg, 3000mg may be employed.

In forming the pharmaceutical composition of the invention in the form of a bilayered tablet, the first layer containing aspirin will also preferably include bulking agents such as lactose, microcrystalline cellulose, wood cellulose, corn starch, modified corn starch, calcium phosphate, sugar, dextrose, mannitol or sorbitol. The bulking agent will be present in an amount from about 1 to about 90%, preferably from about 5 to about 85% by weight of the first layer containing aspirin.

The first layer may also include a tabletting lubricant, such as zinc stearate, magnesium stearate, calcium stearate, talc, carnauba wax, stearic acid, palmitic acid or hydrogenated vegetable oils and fats, in an amount within the range from about 0.01 to about 4%, and preferably 0.02 to about 2% by weight of the first layer.

The second layer of the bilayered tablet containing betaine agent will usually include a bulking agent such as lactose, microcrystalline cellulose, modified corn starch, calcium phosphate or other bulking agent as set out above for the first layer, in an amount within the range from about 1 to about 90%, preferably from about 5 to about 85% by weight of the second layer. In addition, the second layer may include a binder such as corn starch, pregelatinized starch, polyvinyl pyrrolidone (PVP), hydroxypropylmethyl cellulose (HPMC), ethyl cellulose, cellulsoe acetate and the like, in an amount within the range from about 0.5 to about 20%, preferably from about 1 to about 10% by weight of the second layer, and a tabletting lubricant such

as magnesium stearate, zinc stearate, or other lubricant as set out above with respect to the first layer in an amount from about 0.01 to about 4%, preferably from about 0.02 to about 2% by weight of the second layer.

The buffering agents present in the second layer may include conventional acid buffers such as calcium carbonate, magnesium oxide, magnesium carbonate, magnesium hydroxide, aluminum hydroxide, dihydroxyaluminum sodium carbonate, aluminum magnesium hydroxide sulfate or aluminum hydroxide magnesium carbonate co-dried gel, or mixtures of one or more thereof, in amounts as needed to insure that the aspirin will be sufficiently buffered to inhibit GI side effects. Thus, amounts of buffering agent within the range from about 10 to about 1000 mg, preferably from about 50 to about 500 mg will be employed depending upon the amount of aspirin present in the first layer.

In forming the bilayered tablet of the invention, the first layer containing aspirin may be prepared by conventional wet granulation or dry granulation (compaction) techniques.

The second layer containing betaine and buffers may be prepared by conventional wet granulation or dry granulation (compaction) techniques.

The first and second layers may then be compressed and combined to form a bilayered tablet employing conventional bilayer tabletting equipment.

Other conventional ingredients which may optionally be present in either of the two layers include preservatives, stabilizers, anti-adherents or silica flow conditioners or glidants, such as Syloid brand silicon dioxide as well as antioxidants such as Vitamin E, Vitamin C, and folic acid, Vitamin B.sub.6 and Vitamin B.sub.12.

The bilayer tablet of the invention may also include an outer protective coating layer which may comprise from 0 to about 15% by weight of the bilayer tablet. The outer protective coating layer which is applied over the

bilayered tablet may comprise any conventional coating formulations and will include one or more film-formers or binders, such as a hydrophilic polymer like hydroxy-propylmethyl cellulose (HPMC) and a hydrophobic polymer like ethyl cellulose, cellulose acetate, polyvinyl alcohol-maleic anhydride copolymers, acrylic copolymers, beta.-pinene polymers, glyceryl esters of wood resins and the like, and one or more plasticizers, such as polyethylene glycol, triethyl citrate, diethyl phthalate, propylene glycol, glycerin, butyl phthalate, castor oil and the like.

The film formers are applied from a solvent system containing one or more solvents including water, alcohols like methyl alcohol, ethyl alcohol or isopropyl alcohol, ketones like acetone, or ethylmethyl ketone, chlorinated hydrocarbons like methylene chloride, dichloroethane, and 1,1,1-trichloroethane.

The pharmaceutical composition of the invention in the form of a cored tablet wherein the aspirin forms the core, and betaine plus buffering agent are present in a surrounding coat layer, may be prepared employing conventional cored tablet technology. Thus, the aspirin containing core (including excipients and other ingredients as described for the first layer in the bilayered tablet of the invention) may be formed in a manner similar to the first layer of the bilayered tablet as described hereinbefore. The buffering layer containing betaine as well as excipients and other ingredients (as described hereinbefore for the second layer of the bilayered tablet of the invention) may be compressed onto the core as a dry coat.

The so-formed cored tablet may be coated with an outer protective coating layer as described above for the bilayered tablet.

Another embodiment of the pharmaceutical composition of the invention is formed of tablets or capsules containing a mixture of enteric coated aspirin granules, and a betaine may be in the form of a tablet or capsule.

The aspirin granules can be coated with conventional enteric polymers coatings in aqueous or non-aqueous systems. For example, Eudragit L-30D-55 (acrylic acid copolymers-Rohm Pharma) (5 to 25% solids) containing 10 to 15% of diethylphthlate (w/w) as plasticizer can be used in an aqueous system.

Other conventional enteric polymer coating systems may be employed such as Eudragit R and S series resins, (acrylic acid copolymers-Rohm Pharma), cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, hydroxypropylmethylcellulose acetate succinate, cellulose acetate maleate, cellulose acetate succinate and the like, and a suitable plasticizer such as triethyl citrate, diethyl phthalate, tributyl citrate, triacetin, dibutyl phthalate, dibutyl sebicate, Myvacet 940, and other commonly used plasticizers as may be suitable for particular enteric polymers can be used. It will be appreciated that any polymer with suitable plasticizer can be used in aqueous or non-aqueous system to form an enteric coating on the aspirin granule or particle.

In another embodiment of the pharmaceutical composition of the invention, the enteric coated aspirin granules described above may be further coated with an outer protective finishing coat or layer as described hereinbefore.

The double coated aspirin granules can be mixed with a betaine such as Betaines powders or granules and the mixture can be encapsulated or tableted as described above.

In yet another embodiment of the pharmaceutical composition of the invention, aspirin is enteric coated as described above and the betaine can optionally be enteric coated. The Betaines can be coated in the form of pure drugs or after spheronization or agglomeration. The particles for coating do not need to be perfectly spherical. These could be rods or irregular particles. The enteric coated particles of the two drugs (aspirin and betaine) can be tableted or encapsulated together. As described above, appropriate excipients (fillers, binders, disintegrants, and lubricant, etc.) can be used to facilitate

tabletting. This betaine/aspirin combination will minimize side effects of aspirin, and eliminate chemical incompatibility.

If, aspirin side effects are not an issue, especially at lower (e.g., 80 mg) aspirin dosages, then aspirin granules (including uncoated aspirin) can be mixed with betaine powder or granules for tabletting or for encapsulating.

In yet another embodiment, aspirin granules can be mixed with enteric coated particles of betaine and the mixture can be tableted or encapsulated or the two granules can be encapsulated in two shots in the same capsule shells.

In carrying out the method of the present invention, the pharmaceutical composition of the invention containing the combination of the betaine and aspirin may be administered to mammalian species, such as monkeys, dogs, cattle, livestock, cats, rats, humans, etc., and, as described hereinbefore, may be incorporated in a tablet or capsule. The above dosage forms will also include the necessary carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants such as Vitamin C and Vitamin E, as well as Vitamin B.sub.6, Vitamin B.sub.12, folic acid, sodium bisulfite, and the like.

The dose administered must be adjusted according to age, weight and condition of the patient, as well as the route of administration, dosage form and regimen and the desired result.

The compositions described above may be administered in the dosage forms as described above in single or divided doses of one to four times daily. It may be advisable to start a patient on a low dose combination and work up gradually to a high dose combination.

Tablets of various sizes can be prepared, e.g., of about 2 to 2000 mg in total weight, containing the active substances in the ranges described above, with the remainder being a physiologically acceptable carrier of other materials according to accepted pharmaceutical practice. These tablets can, of course,

be scored to provide for fractional doses in some cases. Gelatin capsules can be similarly formulated.

Liquid formulations can also be prepared by dissolving or suspending one or the combination of active substances in a conventional liquid vehicle acceptable for pharmaceutical administration so as to provide the desired dosage in one to four teaspoonsful.

Such dosage forms can be administered to the patient on a regimen of one to four doses per day.

In general, formulating the compositions, as described herein, the active substances, in the amounts described above, are compounded as described herein (according to accepted pharmaceutical practice) with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in the particular type of unit dosage form.

Illustrative of the excipients which may be incorporated in tablets are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate or cellulose; a disintegrating agent such as corn starch, potato starch, alginic acid, sodium starch glycolate or the like; a lubricant such as stearic acid, zinc stearate or magnesium stearate; a sweetening agent such as sucrose, aspartame, lactose or saccharin; a flavoring agent such as orange, peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. As indicated, various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compounds, water, alcohol or the like as the carrier, glycerol as solubilizer, sucrose as sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange.

Some of the active substances described above form commonly known, pharmaceutically acceptable salts such as alkali metal and other common basic salts or acid addition salts, etc. References to the base substances are therefore intended to include those common salts known to be substantially equivalent to the parent compound.

The formulations as described above will be administered for a prolonged period, that is, for as long as the potential for cardiovascular events and disease including coronary artery disease and/or cerebrovascular disease remains or the symptoms continue. Sustained release forms of such formulations which may provide such amounts daily, biweekly, weekly, monthly and the like may also be employed. A dosing period of at least 10 days are required to achieve minimal benefit.

The following examples illustrate various aspects of the present invention. They are not to be construed to limit the claims in any manner whatsoever.

Parenteral formulations

Salicylic acid is used in the form of its acetyl derivative to a large extent as an analgesic. Although this acetyl derivative, known as Aspirin, was originally developed to reduce disturbing side effects of the salicylic acid already known earlier, nevertheless, it is affected by several properties which limit its possibilities of use. Above all, its low water solubility, in particular in an acid medium, for example, in gastric juice, is one of these unfavourable properties. With oral administration of aqueous solutions, the low solubility can lead to the precipitation of the active ingredient in the stomach. This effect is undesirable not only in individuals with a sensitive or previously damaged gastric mucous membrane, since it can lead to serious side effects in these individuals, but it quite generally slows down the resorbtion and, thus, also the beginning of the therapeutic action.

Because of its limited solubility in water (about 0.3%), acetylsalicylic acid can be administered practically only orally, but not parenterally, for example, intravenously, intraperitoneally or topically. But precisely because of the quick onset of action and/or the gentle treatment of the gastrointestinal tract, a parenteral administration would often be desirable.

In one embodiment the object of the invention is to provide a salicylic acid derivative, which is also readily water-soluble in the acid range, is easily resorbed, exhibits a lowest possible toxicity and can be administered both enterally and parenterally or topically and shows a quickly starting therapeutic action in all forms of administration.

In one embodiment this object is achieved by Betaines/aspirin combinations of the invention. In effect, high concentrations of betaine in water provide for a completely new super-solvent for salicylic acid. Even more impressive is the improved solubility of salicylic acid. Up to 5% salicylic acid can be dissolved in a cold concentrated betaine solution. That figure is 15 times the amount of salicylic acid that can be dissolved in pure water.

For the parenteral formulations, lipidic betaines, betaines lipids and glycine betaine monohydrate, anhydrous, hydrochloride and its salts pharmaceutically acceptable will be preferred.

In one embodiment, the injectable combinations of the invention will be useful for the treatment of pain, inflammation, and fever rheumatoid arthritis, osteoarthritis, juvenile and adult forms of arthritis, gout, dysmenorrhea, muscle pains, and dental pain.

As the pH of an aqueous glycine betaine solution is comprised between about 6 and about 7, an injectable solution (preferably for a subcutaneous injection) can be prepared by mixing solid glycine betaine with water (sterilized and possibly demineralised) *and salicylic acid and derivatives*. The glycine betaine can be in the form of a powder (lyophilized powder) placed in a vial, water is then added to said vial for the preparation of the solution to be

injected. If necessary, some acid (such as hydrochloric) can be added to the solution or to the water to be mixed with the powder.

Acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof and Betaines, preferably glycine betaine in an injectable dosage form can be in a pressurized dosage form, such as an air pressurized dosage form. Subcutaneous and intravenous injectable forms of the combinations, are preferred. In such injectable forms the amount of Betaines will be at least twice the amount of acetylsalicylic acid, salicylic acid, salicylate, pharmaceutical derivatives thereof and mixtures thereof calculated as acetylsalicylic acid. The combination injectable forms are for example aqueous solution containing 0.1 to 95% by weight glycine betaine, advantageously from 0.5 to 30%, preferably from 10 to 20%. The injectable form has a pH for example comprised between 5 and 8.5, advantageously from 6 to 7.5, preferably from 6 to 6.5. When the injectable form is prepared by mixing glycine betaine (as a solid form or as a powder form) acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, the pH of the solution is about 6 – 6.5.

When acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof and Betaines, preferably glycine betaine, are administered by injection, the combination can be present in an individual vial suitable for single or repeated administrations.

When acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof and Betaines, preferably glycine betaine are administered by injection or slow infusion the combination can be present as a solution in a flexible bag (Baxter), for example a flexible bag (Baxter) for intravenous administration of a saline solution, or a physiological solution, or a blood transfusion Baxter.

The invention relate thus also to a bag (flexible bag or Baxter) for subcutaneous administration (preferably intravenous administration) containing a solution suitable for subcutaneous administration. As more specific example, the bag or Baxter contains blood or a blood derivative or a blood portion and acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof and Betaines, preferably glycine betaine for parenterally administration.

A subject matter of the invention is thus a pharmaceutical combination comprising an effective amount of betaine (preferably glycine betaine) and an effective amount of another active agent for the prevention or treatment of troubles.

Betaine is preferably used as therapeutically effective agent in said combination.

EXAMPLES

Example 1

A bilayered tablet containing aspirin in a first layer and betaine in a second layer as described below may be prepared as follows.

General Formula:

	Amount or %
First Layer:	in First Layer
Aspirin granulation	5 mg-300 mg
Lactose/microcrystalline	qs
Cellulose granulation*	
Zinc Stearate	0.1%-0.5

Second Layer:	Amount in Second Layer
Calcium Carbonate	5 mg-250 mg
Magnesium Oxide	5mg-100 mg
Magnesium Carbonate	2 mg-50 mg
Corn Starch	5 mg-50 mg
Betaine	100 mg-800 mg
Magnesium stearate	0.2%-0.5%

*This is an inert granulation just for the purpose of bulking, if necessary. This will contain 50%-90% lactose anhydrous, 10%-50% microcrystalline cellulose, and 0.1%-0.5% zinc stearate. These ingredients are blended, and appropriate size granules are prepared by conventional dry granulation process. (This being just an inert granulation, any other excipient can be used to prepare granules for bulking by dry or wet granulation processes, # so that the granules do not have alkalizing agent and also do not contain excessive moisture and are compatible with aspirin granules. These bulking granules must have enough compatibility to allow compression of two layer tablets).

Procedure

The aspirin granulation in the first layer is blended with sufficient quantity of the lactose/microcrystalline cellulose granulation as necessary to bulk up in order to have sufficient granulation to compress a satisfactory layer. The aspirin granules along with the bulking granules are blended with zinc stearate as a lubricant. Zinc stearate can be replaced with other non-alkaline lubricants, i.e., Lubritab.®. or other high melting point hydrogenated powdered waxes

Ingredients in the second layer are wet granulated using starch paste or other

wet granulating materials, for example, PVP or HPMC, or can be dry granulated by compaction. The granules can be sized and lubricated. The two tablet layers are compressed using appropriate conventional tools and a suitable bilayer tabletting press, to form the bilayered tablet of the invention.

The quantity of the buffering agents used in the second layer can be adjusted as necessary to minimize gastrointestinal side effects. It should be understood that these buffering agents can be replaced with other suitable buffering agents, if desired.

The so-formed bilayered tablets may be coated with HPMC (hydroxypropylmethylcellulose) or commercially available Opadry.®. clear or Dri Klear.®. (HPMC) or any of these with any desired color. This coat is not limited to HPMC based coats only. Polymers, i.e., Eudragit E30D (acrylic acid copolymer) and others can also be used to give the tablets a finishing coat.

Coating Formula (example):

Opadry .®. clear 10%-30%

Purified water qs

Procedure

Opadry.®. is dispersed in water to prepare a dispersion of 10%-30% solids*. This dispersion is used for coating the above tablets using conventional coating equipment. The coating of 0.2%-2% or any desired level (based on the weight of the finished coated bilayered tablet) can be applied to the bilayered tablet employing conventional techniques.

*Antifoam emulsion at a level of 0.1 to 2% of solids, can also be included in the formulation.

The so-formed tablets provide maximum benefits while minimizing drug interaction and other undesirable side effects.

It will be understood that betaine contained in the buffered layer of the bilayered tablet of the invention may be replaced with equivalent amounts of lipidic betaines and/or betaine lipids.

Example 2

Tablets or capsules containing enteric coated aspirin and a betaine, which preferably is anhydrous betaine, monohydrate betaine, having the following composition are prepared as described below.

General Formula:

Aspirin particles	5 mg-325 mg
Eudragit L-30D-55	qs
Diethyl Phthalate	qs
Betaines, Desired Dose	

Procedure

Aspirin particles are coated with enteric polymers in aqueous or non-aqueous systems. Eudragit L-30D-55 containing 10%-15% of diethyl phthalate (w/w) is used in an aqueous system. The coating suspension is prepared having solid contents of 10%-30%.

To prepare the coating suspension, diethyl phthalate is added to the Eudragit L-30D-55 and the contents stirred till diethyl phthalate is completely

dissolved. This is diluted with water to obtain the suspension with desired solid contents. Using this enteric coating suspension, the aspirin particles are coated in a fluid bed coating system using a Wurster insert or with top spray coating, so that aspirin particles of enteric quality can be produced. The enteric coated particles are mixed with betaine powders or granules and the mixtures are encapsulated or tableted using appropriate excipients (fillers, binder, disintegrants, and lubricants). Any of the listed betaine can be selected at its desired dose level along with the desired aspirin dose.

The Betaines can also be granulated, and the betaine granules and the enteric coated aspirin granules can be filled separately into the same capsule shell. Betaine granules can be prepared by dry or wet granulation processes, using suitable conventional excipients as is well known in the pharmaceutical field.

The above formulations provide maximum benefit while minimizing undesirable side effects and incompatibilities.

Example 3

A cored tablet containing an aspirin core and a buffered coating thereon containing a betaine having the following composition is prepared as described below.

General Formula:

	Amount or %
Core Layer:	in Core Layer
Aspirin granulation	10 mg-325 mg
Lactose/microcrystalline	qs
Cellulose granulation*	
Zinc Stearate	0.1%-0.5
	Amount in Second

Outer Layer:	Layer
Calcium Carbonate	5 mg-250 mg
Magnesium Oxide	5 mg-100 mg
Magnesium Carbonate	5 mg-50 mg
Corn Starch	5 mg-50 mg
Betaine	100 mg-1000 mg
Magnesium stearate	0.2%-0.5%
Filler/Binder**	qs

*This is an inert granulation just for the purpose of bulking, if necessary. This will contain 50%-90% lactose anhydrous, 10%-50% microcrystalline cellulose, and 0.1%-0.5% zinc stearate. These ingredients are blended, and appropriate size granules are prepared by conventional dry granulation process. (This being just an inert granulation, any other excipient can be used to prepare granules for bulking by dry or wet granulation processes, # so that the granules do not have alkalizing agent and also do not contain excessive moisture and are compatible with aspirin granules. These bulking granules must have enough compatibility to allow compression of two layer tablets).

**The Filler/Binder may be any known fillers or tablet binders, such as lactose, microcrystalline cellulose, modified starch, calcium phosphate and the like.

Procedure

The aspirin granulation for the core is blended with sufficient quantity of the lactose/microcrystalline cellulose granulation as necessary to bulk up in order to have sufficient granulation to compress a satisfactory core. The aspirin granules along with the bulking granules are blended with zinc stearate as a lubricant. Zinc stearate can be replaced with other non-alkaline lubricants, i.e., Lubritab.®. or other high melting point hydrogenated powdered waxes.

Ingredients for the outer layer are wet granulated using starch paste or other wet granulating materials, for example, PVP or HPMC, or can be dry

granulated by compaction. The granules can be sized and lubricated. The dry coated tablets can be compressed using appropriate tools and a suitable dry coating tabletting press.

The quantity of the buffering agents used in the outer layer can be adjusted as in Example 1. Other known buffering agents may be used as well.

The modes, the methods, the uses and the formulations as described in the application PCT/BE 02/ 00013 of one of the applicants, the scope of which is incorporated here by reference, are incorporated and also suitable for the Betaines alone and for the Betaines / aspirin and derivatives combinations in the scope of the present invention.

Example 4

Pharmacological activity in animal studies

Appratus and methods

Material

ASPEGIC® injectable aspirin Synthelabo France

Betaine monohydrate, BETAFIN ® (Finnsugar Bioproducts, CULTOR, Helinski)

Rats Wistar, males, weight between 250 and 300 grams

Sodium Thiopental

Aggregometer CHRONOLOG COULTRONIC S.A. France

ADP Laboratoires Stago France

Methods

Aspirin dosage

The doses of 100 mg /kg and 50 mg /kg of aspirin used in these experiments are the doses known to be antithrombotic in rat in this model. Due to rat known resistance to aspirin these high doses are necessary and represent at least 10 to 25 fold the antithrombotic doses needed in other species in experimental thrombosis. Due to this specificity of rat the ratios betaine/aspirin used in these experiments don't reflect the ratio to be used in other species. In human clinical practice for instance, an orally daily dose of 75 to 300 mg, i.e. 1 to 5 mg/ kg is known to be antithrombotic. At this human dose (1 to 5 mg/ kg), betaine at 3 to 15 mg/kg or more, will be in accordance with the invention.

Aggregation tests

The aggregation is made in accordance to the methods Cardinal & Flower. Pharmacol. Method.1980 and to American Journal of Clinical Pathology, 1989; 92: 676-679. Sureney. JD. Whole Blood aggregometry.

After a keeping period of 8 days, the rats are subjected to a fasting for 12 hours. Betaine is subcutaneous injected one hour before blood sampling. The rats are then anaesthetised with sodium Thiopental administered at a dose of 200 mg/Kg and the blood samples are taken by intracardiac puncture on a trisodium citrate solution (1 volume of solution at 3, 8 % citrate for 9 volumes of blood).

Induced haemorrahgic time IHT

(E. Dejana. Bleeding time in rats . Thrombosis. Rech. 1982)

Blood samples are made before the test. The tail of anaesthetised rat, is dipped for 5 minutes in a water bath at 37°C so as to provoke a dilatation of the peripheral vessels which are removed and cut at the end, the chronometer

being started. The IHT is defined as being the time period comprised between the cutting of end tail and the end of the haemorrhage or bleeding. The end of haemorrhage is defined as the time where the last drop of blood is removed from the tail and no other drop is seen during 180 seconds. The substances were subcutaneously administrated 60 minutes prior to the tail cut.

Principle of laser-induced thrombosis
(Seiffge D. et al., 1989; Weichter W. et al., 1983)

In this model, lesion of the vascular wall is induced by a laser beam. This beam causes a limited lesion of the vascular endothelium (only 1 to 2 cells are destroyed). This laying bare of the sub-endothelium, which is a thrombogenetic surface, results in the adherence of platelets via glycoproteins. This adherence of platelets is followed by their activation, they form pseudopods and secrete the content of their granules. This activation results in the appearance of glycoprotein binding sites which are necessary for the aggregation of the platelets between them and for platelet adhesion to the thrombogenic surfaces. This lesion is induced in the mesenteric microcirculation of the rat. It is immediately followed by the formation of a thrombus (in a few seconds). This thrombus, which rapidly enlarges under the influence of the blood flow, embolises before being formed again.

A. Aspirin 100 mg/kg / betaine 5 mg /kg combination

The aim of the study was to investigate the effect of betaine on various parameters in combination with aspirin.

Experimental protocol.

The substances were subcutaneously injected 1 hour before the tests.

Laser experiment
(Saline control = 2 to 3 laser shoots & 5 to 6 emboli)

	Number of laser shoots		Number of emboli	
	ASA 100 mg /kg	ASA 100 mg /kg + betaine 5 mg /kg	ASA 100 mg /kg	ASA 100 mg /kg + betaine 5 mg /kg
Rat 1	4	4	0	0
Rat 2	3	4	1	0
Rat 3	3	4	1	0
Rat 4	3	4	2	0
Rat 5	3	4	2	0
Rat 6	-	4	-	1
Mean	**3,2 ± 0,45**	**4 ± 0**	**1,2 ± 0,84**	**0,17 ± 0,41**

Aggregation tests (ADP 5 µM in final concentration)

(Saline control = amplitude ± 15 ohm & velocity ± 13 ohm /min)

	Amplitude (ohm)		Velocity (ohm /min)	
	ASA 100 mg /kg	ASA 100 mg /kg + betaine 5 mg /kg	ASA 100 mg / kg	ASA 100 mg /kg + betaine 5 mg /kg
Rat 1	2	2	4	2
Rat 2	3	0	4	0
Rat 3	2	2	3	3
Rat 4	4	0	5	1
Rat 5	2	2	3	3
Rat 6	-	2	-	3
Mean	**2,6 ± 0,89**	**1,33 ± 1,03**	**3,8 ± 0,84**	**2 ± 1,26**

Induced haemorrhage (tail cut, Dejana)

(Saline control = ± 110 seconds)

	IHT (seconds)	
	ASA 100 mg /kg	ASA 100 mg /kg + betaine 5 mg /kg
Rat 1	340	340
Rat 2	330	320
Rat 3	340	370
Rat 4	345	345
Rat 5	420	360
Rat 6	-	320
Mean	**355 ± 36,74**	**342 ± 20,43**

Discussion.

Betaine in combination with ASA had a little effect on induced haemorrhage. Surprisingly the combination shows better antithrombotic (more laser shoots & fewer emboli) effect than ASA alone, this being confirmed in the aggregation test. Betaine is potentialising aspirin effect, suggesting its possible activity on a different mechanism than aspirin. In this model the combination betaine / ASA showed better results than dipyridamole / aspirin combination suggesting a future clinical development.

B. Aspirin 50mg/kg / betaine 10 mg /kg combination

Experimental protocol.

Betaine monohydrate Finnsugar, ASA Synthelabo

The substances were subcutaneously injected 1 hour before the tests.

Laser experiment

Saline control NaCl 0, 9% subcutaneously 1 hour before experiments, duration of embolisation is expressed in minutes

	Number of laser shoots	Number of emboli	Duration of embolisation
Rat 1	2	6	3
Rat 2	2	5	2
Rat 3	1	6	3
Rat 4	1	7	4
Rat 5	2	5	2
Rat 6	1	6	4
Mean	1,5	5,83	3

Treated groups, ASA 50 mg /kg (n = 6 rats) or ASA 50 mg /kg + Betaine 10 mg/kg (n = 6 rats) are subcutaneously administrated 1 hour before experiments

	Number of laser shoots		Number of emboli		Duration	
	ASA 50 mg /kg	ASA 50 + Betaine 10	ASA 50	ASA 50 + Betaine 10	ASA 50	ASA 50 + Betaine 10
Rat 1	2	3	4	1	3	0
Rat 2	3	2	3	1	2	0
Rat 3	2	4	3	0	3	0
Rat 4	2	4	4	0	4	0
Rat 5	3	3	3	1	3	0
Rat 6	2	4	3	0	4	0
Mean	2,33	3,33	3,33	0,5	2,5	0

Aggregation tests (ADP 5 μM in final concentration)

Amplitudes are expressed in Ohm and Velocities in Ohm /min.

	Saline		ASA 50		ASA 50 +Betaine 10	
	Amplitude	velocity	Amplitude	velocity	Amplitude	velocity
Rat 1	15	14	8	7	2	2
Rat 2	16	14	9	8	0	0
Rat 3	15	13	10	9	1	0
Rat 4	17	13	7	6	0	0
Rat 5	16	15	9	7	2	3
Rat 6	15	12	8	7	2	1
Mean	15,67	13,5	8,5	7,33	1,17	1

Tail cut induced haemorrhage (Dejana)

	Saline	ASA 50	ASA 50 + Betaine 10
Rat 1	98	183	114
Rat 2	123	192	120
Rat 3	107	176	127
Rat 4	102	163	174
Rat 5	114	185	111
Rat 6	107	180	119
Mean	108,5	179,83	127,5

Discussion.

Betaine in combination with ASA had a significant effect on induced haemorrhage while preserving the antithrombotic effect. The combination reducing by 2 times the therapeutic dose in rats (50 mg/kg instead of 100 mg/kg) shows better antithrombotic (more laser shoots & fewer emboli) effect than ASA alone at 100 mg/ kg, this being confirmed in the aggregation test. The combination of the invention clearly allows to reduce the effective dose of aspirin while obtaining a significant improvement in the therapeutic effect. Betaine is potentialising aspirin effect, suggesting its possible activity on a different mechanism than aspirin. The two molecules act in a synergistic manner to prevent thrombosis in this model. The combination betaine/ ASA

showed better results than dipyridamole/ aspirin combination suggesting future clinical development.

These results show that glycine betaine maintains the tail cut bleeding time within the values of the negative control. In addition to its anti-thrombotic activity, the combination does not result in any risk of haemorrhage compared with the positive controls.

Treatment with asa/betaine combination completely inhibits the thrombo-embolic complications which are initiated by laser firings. In fact, treatment with asa/betaine combination before laser firings decreases the vascular adherence of platelets and the aggregation thereof.

Treatment with asa/betaine combination completely inhibits thrombo-embolic complications. In fact, treatment with asa/betaine combination before the induction of thrombosis exhibited a high antithrombotic potential with regard to all the parameters involved in thrombus formation process.

According the results presented above, this drug also exhibits anticoagulant, anti-aggregant and fibrinolytic indications. The demonstrated innocuousness of this combination enables long-term treatments to be considered which do not necessitate biological monitoring.

Interest in the use of asa/betaine combination is based on the fact that it acts at several levels of haemostatis, i.e. it acts on platelet aggregation, coagulation and fibrinolysis. This activity is durable and prevents repeated administration, which constitutes a considerable improvement in relation to existing treatments. The administration of betaine/asa does not induce any haemorrahgic risk or other side effects (e.g. heparin-induced thrombopenia), which constitutes a major advance in antithrombotic therapy.

C. Aspirin 100 mg/kg / betaine 10 mg /kg combination

Experimental protocol.

Betaine monohydrate Finnsugar, ASA Sigma Aldrich

The substances were subcutaneously injected 1 hour before the tests.

Laser experiment

Saline control NaCl 0, 9% subcutaneously 1 hour before experiments, duration of embolisation is expressed in minutes

	Number of laser shoots	Number of emboli	Duration of embolisation
Rat 1	2	6	3
Rat 2	2	5	2
Rat 3	1	6	3
Rat 4	1	7	4
Rat 5	2	5	2
Rat 6	1	6	4
Mean	**1,5**	**5,83**	**3**

Treated groups, ASA100 mg /kg (n = 6 rats) or ASA100 mg /kg + Betaine 10 mg/kg (n = 6 rats) are subcutaneously administrated 1 hour before experiments

	Number laser shoots		Number of emboli		Duration	
	ASA100 mg /kg	ASA100 + Betaine 10	ASA100	ASA100 + Betaine 10	ASA100	ASA100 + Betaine 10
Rat 1	2	4	2	0	1	0
Rat 2	3	4	2	0	1	0
Rat 3	3	4	1	0	0	0
Rat 4	3	4	1	0	0	0
Rat 5	4	4	0	0	0	0
Rat 6	3	4	2	0	1	0
Mean	3	4	1,33	0	0,5	0

Aggregation tests (ADP 5 µM in final concentration)

Amplitudes are expressed in Ohm and Velocities in Ohm /min.

	Saline		ASA100		ASA100 +Betaine 10	
	Amplitude	velocity	Amplitude	velocity	Amplitude	velocity
Rat 1	15	14	3	4	0	0
Rat 2	16	14	3	3	0	0
Rat 3	15	13	2	4	1	2
Rat 4	17	13	2	2	0	0
Rat 5	16	15	1	0	0	0
Rat 6	15	12	4	4	1	0
Mean	15,67	13,5	2,5	2,83	0,33	0,33

Tail cut induced haemorrhage in seconds (Dejana)

	Saline	ASA100	ASA100 +Bet. 10
Rat 1	98	367	270
Rat 2	123	413	290
Rat 3	107	388	285
Rat 4	102	397	240
Rat 5	114	392	265
Rat 6	107	368	250
Mean	108,5	387,5	266,7

Discussion.

Betaine in combination with ASA had a significant effect on induced haemorrhage. The combination shows better antithrombotic (more laser shoots & fewer emboli) effect than ASA alone, this being confirmed in the aggregation test. Betaine is potentialising aspirin effect, suggesting its possible activity on a different mechanism than aspirin. In this model the combination betaine / ASA showed better results than clopidogrel/ aspirin combination confirming that this new combination in that ratio may be useful for parenterally administration in future acute clinical situations (i.e.

angioplasties, prosthesis, stents placement, hip surgery, prosthetic heart valves, arterial grafts and replacement surgery).

Example 5 :

Pharmacological activity in human ex vivo test

Parallel plate chamber studies

The synergistic effect of asa/betaine on platelet adhesion is studied by perfusing blood on thrombogenic surface such as collagen under shear stress conditions.

Experimental design

Effect of betaine, aspirin or betaine/aspirin combination on platelet adhesion and thrombus formation on collagen coated surface.

We studied the effect of asa/betaine on platelet adhesion and thrombus formation under laminar flow at high shear stress (60 dynes /cm^2) on a collagen coated surface. This high shear stress mimics stenosed arteries.
The blood of four fastened healthy subjects was sampled on day 1 as to determine basal values and betaine effect when added in vitro (table1), then the subjects were orally administrated a daily oral bolus dose of 350 mg of ASA during 3 days and their blood sampled on day 3 as to determine ASA effect and ASA + betaine effect when betaine is added in vitro to aspirinized blood at different concentrations (table2).

Citrated human aspirinized blood was pre-labelled with mepacrine and exposed to vehicle alone or betaine (20, 40, and 80 µg/ml) for 20 minutes before perfusion.

Effect of asa/betaine on platelet adhesion and thrombus formation on collagen coated surface.

The effect of asa/betaine has been evaluated by performing experiments 60 dynes/cm^2 witch mimics arterial thrombosis. Before perfusion citrated whole blood was incubated with saline alone or betaine at concentration of 20, 40, and 80 µg /ml for 20 minutes and then perfused on collagen at 60 dynes /cm^2. At the end of 2 minutes of perfusion, cells were fixed and the area covered by thrombi was calculated by analysis of fluorescent thrombus images acquired by confocal microscopy.

Experimental Methods

Collagen coated slides. Glass slides were with collagen (200µg/ml; equine collagen), placed for 1 hour in a water bath at 37°C, and the collagen gel allowed to evaporate for 30 minutes under a ventilated hood. Perfusion with citrated whole blood was started and continued for 2 minutes, then blood was withdrawn from the circuit, and platelet thrombi adherent to the collagen coated surface were fixed and stained with May Grunwald-Giemsa.
Images of platelet thrombi were digitised using a light microscope connected to a computer-based image analysis system. The surface occupied by thrombi and mean thrombus area were evaluated by automatic edge detection using built-in specific functions of a software image.

Platelet adhesion and thrombus formation. Platelet adhesion assay was performed according to the described previously (Alevriadou et al.,1993; Boccardo et al.,1997) with minor modifications. Blood collected on citrate (3,8 %) was perfused through a flow chamber using a syringe pump. A flow chamber thermostatized to 37°C was used in which one surface of the perfusion channel is a glass slide coated with equine collagen.

Flow chamber. The chamber dimensions (30 mm long, 1mm large, and 150 μm in the thickness) allow to obtain a wide range of shear rate low flow rates of blood.

Adhesion assay collagen.

Citrated whole blood was incubated with the fluorescent dye mepacrine 10 μM (quinacrine dihydrochloride BP; Sigma Chemical, St Louis. MO). Mepacrine concentrated in the dense granules of platelets and in the granules of leukocytes and, at this concentration has no effect on normal platelet function (3). Any fluorescence within the erythrocytes is quenched by haemoglobin. Blood was pre-incubated 5 minutes at 37°C before perfusion. The system was filled with PBS pH 7.3, then perfusion with blood was started and maintained at the constant flow rate of 1500 sec^{-1} (corresponding to a shear stress of 60 dynes/cm^2). After 2 min, perfusion was stopped and the slide with the collagen monolayer was dehydrated and fixed in acetone for 20 minutes.

Images of platelet thrombi on collagen surface were acquired by a confocal inverted laser microscope. The surface occupied by thrombi was evaluated by automatic edge detection using built-in specific functions of a software image.

Results

1) *Effect of betaine on platelet adhesion and thrombus formation on collagen.*

Thrombus formation on collagen coated surface studied at the shear stress of 60 dynes /cm^2 in which Von Willebrand factor is involved. The effect of betaine added to non-stimulated blood perfused on collagen at high shear stress (60 dynes/cm^2) shows a dose-dependent inhibitory activity of asa/betaine. As shown in Table 1, blood treated with betaine at the

concentration of 80 µg/ml showed a significant reduction (- 49 %) in platelet deposition in respect to vehicle. Thrombus formation of blood incubated with asa/betaine at 20 and 40 µg /ml was inhibited by 15 and 41% respectively

2) *Effect of asa/betaine on thrombus formation on collagen.*

Thrombus formation on collagen coated surface studied at the shear stress of 60 dynes /cm^2 in which Von Willebrand factor is involved. The effect of betaine and asa/betaine added to non-stimulated blood perfused on collagen at high shear stress (60 dynes/cm^2) shows a dose-dependent inhibitory activity of asa/betaine. As shown in Table 2, aspirinized blood treated with betaine at the concentration of 80 µg/ml showed a significant reduction (- 63 %) in platelet deposition in respect to vehicle and a significant reduction in respect to aspirin alone (- 51 %) and to betaine alone (- 27 %).

Table 1 :

Subject	saline	betaine 20 µg/ml	betaine 40 µg/ml	betaine 80 µg/ml
1	100586	88746	66852	57544
2	145921	135241	92433	81585
3	165239	122369	89628	76654
4	175692	154425	95645	82579
Mean	**146859**	**125195**	**86139**	**74590**

Data are expressed as area covered by thrombi (pixels/field)

Blood was incubated with saline or betaine for 20 minutes

Table 2:

Subject	saline	ASA + saline	ASA +betaine 20 µg/ml	ASA +betaine 40 µg/ml	ASA +betaine 80 µg/ml
1	100586	72856	65439	59422	45253
2	145921	121321	121822	76754	55114
3	165239	132458	104583	69575	55752
4	175692	122652	106869	85856	62687
Mean	**146859**	**112322**	**99678**	**72857**	**54701**

Data are expressed as area covered by thrombi (pixels/field)

Aspirinized blood was incubated with saline or betaine for 20 minutes

Comments

In this assay we analysed the potential antithrombotic effect of betaine and asa/betaine on thrombus formation under controlled flow conditions. We observed that under shear stress level high enough to mimic the one encountered in the microcirculation, betaine and asa/betaine significantly inhibit platelet deposition and consequent thrombus formation with respect to vehicle-treated blood. The combination asa/betaine was more effective than aspirin alone or betaine. These tests clearly show the synergistic and addictive effect of the 2 molecules, namely betaine and aspirin, when used in combination in an human ex vivo experimental thrombosis model. These results are predictive of clinical efficacy of the claimed combinations.

Uses of the invention

In view of the above specifications, the invention relates thus also to:

- the use of a one "Betaines" mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof for the preparation of a pharmaceutical composition for the treatment or the prevention of troubles bound to one or more glycoproteins, especially to receptor of one or more glycoproteins, preferably to receptors of glycoproteins Ib and IIb IIIa.
- the use of Betaines mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof for the preparation of a pharmaceutical composition for the treatment or the prevention of troubles bound to one or more glycoproteins, especially to receptor of one or more glycoproteins, preferably to receptor of glycoprotein IIb IIIa for inhibiting the platelet aggregation.
- the use of Betaines mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof for the preparation of a pharmaceutical composition for the treatment or the prevention of troubles bound to one or more glycoproteins, especially to receptor of one or more glycoproteins, preferably to receptor of glycoprotein IIb IIIa for avoiding the adhesion of cells there between
- pharmaceutical composition comprising insulin and Betaines mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof
- pharmaceutical composition comprising an anti cancerous agent and at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof
- pharmaceutical composition comprising an antibiotic and at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as glycoproteic antagonist agent, in particular as antagonist of the glycoprotein Ib and/ or glycoprotein IIb IIIa, for the preparation of a pharmaceutical composition
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to cancer, in particular to the metastasis of cancerous cells
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to blood circulation, in particular to the blood microcirculation
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to chemotherapy
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of diabetic troubles
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to aging
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to oestrogen oral contraception

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutically active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to extracorporeal blood circulation, in particular to troubles bound to dialysis and to haemodialysis
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to inflammation, in particular internal inflammation troubles
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to bites, in particular to bites of venomous animals,
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to post traumatic shock or post surgical shock,
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to septic shocks,
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to embolism, in particular to cerebral embolism and/or pulmonary embolism
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent

for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to an infract

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutically active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to aneurysm
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to phlebitis
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to angina pectoris
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of thromboses troubles, in particular troubles bound to reocclusion of the vascular system and/or to thrombolysis and/or to angioplasty
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to the use of hemoplastic or haemostatic glues, in particular fibrinogen glue, fibrin glue, collagen glue, thrombin glue
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to pregnancy

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of thromboses troubles, in particular coronary thrombosis and/or venous thrombosis

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of bacterial troubles and/or infectious troubles and/or troubles due to virus and/or troubles due to fungus

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of asthmatic troubles

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to osteoporosis

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as therapeutic active agent for the preparation of a pharmaceutical composition for the treatment or the prevention or the stabilization of troubles bound to graft of skin and/or tissue and/or bone and/or cells

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as antagonist agent for serotinin and/or arachidonic acid and/or epinephrine and/or adrenaline and/or thrombin and/or ristocetine for the preparation of a pharmaceutical composition

- uses as disclosed here before for the preparation of a pharmaceutical form, possibly as a kit, containing an active agent different from Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives

and/or mixtures thereof, for the administration (simultaneous or successive, with the same or different administration path) of said other therapeutic active agent and of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Sweetening composition containing at least a sweetener and at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Sweetening composition containing at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as agent for improving the sweetening property of a sweetener, in particular of a synthetic sweetener

- Preserving process for cells and/or platelets in a medium, in particular in a blood medium or a fraction thereof, in which said medium is added or mixed with Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Device with a surface in contact with fibrin and/or fibrinogen and/or collagen, said surface being made of and being treated with a composition containing at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Artificial device destined to be implanted to a living body with a surface in contact with blood said surface being made of and/or being treated with a composition containing at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Artificial device destined to be implanted to a living body with a surface in contact with living tissue said surface being made of and/or being treated with a composition containing at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Composition containing at least fibrinogen and at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Composition containing at least collagen and at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Process for the treatment of blood or a fraction thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof by osmosis and/or reverse osmosis, in which, before and/or during and/or after the osmosis or reverse osmosis, said blood is added or mixed with at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Process for the treatment of blood or a fraction thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof by centrifugation, in which, before and/or during and/or after the centrifugation, said blood is added or mixed with at least Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof

- Biological material or synthetic material for implant purposes, especially for bone implant, said material being treated with Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, and/or a composition containing such a compound

- Process of treatment of a patient suffering of a trouble cited here above in this specification, in which an effective amount of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof is administered to said patient, so as to treat and/or stabilize said trouble

- Process for preventing a patient to suffer a trouble cited here above in this specification, in which an effective amount of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof is administered to said patient, so as to prevent said trouble,

- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as anti agglutinant agent, for the preparation of a pharmaceutical composition
- use of Betaines, mixtures thereof and acetylsalicylic acid, salicylic acid, salicylates and derivatives and/or mixtures thereof, as blood fluidifying agent, for the preparation of a pharmaceutical composition.

The term" Betaines" as employed herein refers to compounds selected from the group consisting of betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, (preferably glycine betaine n = 1), pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, and/or lipidic betaines and/or betaine lipids.

Aspirin will preferably be employed in the form of salicylic acid acetate also referred to as acetylsalicylic acid.

In one embodiment salicylic acid may be employed.

Salicylate salts may also be employed. In one embodiment betaine salicylate as described in French Patent 1.123 M of 1962 may be employed.

In one embodiment, the combinations of the invention will be useful for the treatment of pain, inflammation, and fever rheumatoid arthritis, osteoarthritis, juvenile and adult forms of arthritis, gout, dysmenorrhea, muscle pains, and dental pain.

In one embodiment mixtures of one or more salicylic acid, acetylsalicylic acid and betaine salicylate may be employed.

In one embodiment, the modes, the methods, the combinations, the uses and the formulations as described in the application PCT/BE 02/ 00013 of one of

the applicants, the scope of which is incorporated here by reference, are incorporated and also suitable for the Betaines/ aspirin and derivatives combinations when formulated or combined with antithrombotic, anti inflammatory, anti cancerous and anti diabetic drugs. The applicants claim the combinations as described in the application PCT/BE 02/ 00013 in its whole content when combined or formulated further to the Betaine/ aspirin and derivatives combinations of the present invention. The applicants claim the methods of treatment as described in the application PCT/BE 02/ 00013 in its whole content, when combined further to the methods of treatment of the present invention.

In one embodiment, the modes, the methods, the combinations, the uses and the formulations as described in the application PCT/BE 02/ 00013 of one of the applicants, the scope of which is incorporated here by reference, are incorporated and also suitable for the Betaines/ aspirin and derivatives combinations when Betaines are formulated in a slow or controlled release forms. The applicants claim the combinations as described in the application PCT/BE 02/ 00013 in its whole content when combined or formulated further to the Betaine/ aspirin and derivatives combinations of the present invention when Betaines are formulated in a slow or controlled release forms. The applicants claim the methods of treatment as described in the application PCT/BE 02/ 00013 in its whole content, when combined further to the methods of treatment of the present invention.

The combinations of the invention may be useful for long term therapies as vascular occlusive diseases, inflammation, cancer and diabetes.

WHAT WE CLAIM IS :

1. Pharmaceutical combination comprising at least:

- A first compound selected among the group consisting acetylsalicylic acid, salicylic acid, and pharmaceutical derivatives thereof, and
- A second compound selected from the group consisting of lipidic betaines, betaines lipids, betaine of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof with the provision that said second compound is different from the first compound,

in which said combination comprises less than 100 mg of said first compound expressed as acetylsalicylic acid, and

in which the amount of second compound is at least 3 times the amount, calculated as acetylsalicylic acid weight, of said first compound.

2. The combination of claim 1, which comprises an amount of said first compound, calculated as acetylsalicylic acid, of less than 85 mg, advantageously of less than 75 mg, preferably of less than 60 mg.

3. The combination of claim 1, which comprises an amount of acetylsalicylic acid or pharmaceutical derivative thereof corresponding to 3 to 80 mg, advantageously from 5 to 75 mg, preferably from 10 to 75 mg calculated as acetylsalicylic acid.

4. The combination of claim 1, in which the amount of second compound is at least comprised between 3 and 100 times the amount calculated as acetylsalicylic acid weight of said first compound, advantageously comprised between 5 and 25 times the amount calculated as acetylsalicylic acid weight of said first compound.

5. The combination of claim 1 as an unitary dose, in which the amount of second compound is 60 times the amount, calculated as acetylsalicylic acid weight, of said first compound.

6. The combination of claim 1, which is prepared at least from a mixture in which at least 50% by weight of the first compound and at least 50% of the second compound are in soluble form.

7. The combination of claim 1 , which is prepared at least from a mixture in which at least 90% by weight of the first compound and at least 90% of the second compound are in soluble form.

8. The combination of claim 1, which is prepared at least from a mixture in which the first compound and the second compound are substantially completely in soluble form.

9. The combination of claim 1, which the second compound is at least in a controlled release form.

10. The combination of claim 1, which the first compound is at least partly in an immediate release form.

11. The combination of claim 1, which comprises dry particles, especially micro particles, prepared by drying a mixture in which the first compound and the second compound are partly in a soluble form.

12. The combination of claim 1, in which the first compound and the second compound are combined in the form selected from the group consisting of a matrix, a gel, an hydrogel, a wax and a porous carrier, a bilayered tablet and combination thereof.

13. The combination of claim 1, which further comprises at least one compound reacting in presence of water so as to prepare substantially immediately a solution or suspension of first compound and second compound.

14. The combination of claim 1 in which the second compound comprise at least glycine betaine monohydrate.

15. The combination of claim 1 in which the second compound comprise at least glycine betaine anhydrous.

16. Pharmaceutical unit dosage form comprising at least a pharmaceutical combination containing at least:

A first compound selected among the group consisting acetylsalicylic acid, salicylic acid, and pharmaceutical derivatives thereof, and

A second compound selected from the group consisting of lipidic betaines, betaines lipid, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, or a pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound, in which the combination is prepared from a mixture in which the first compound and the second compound are partly in a soluble form.

17. The pharmaceutical form according to claim 16, which comprises less than 500 mg, advantageously less than 300 mg, preferably less than 100 mg of said first compound expressed as acetylsalicylic acid.

18. The pharmaceutical form according to claim 16, which the amount of second compound is at least 3 times the amount by weight of said first compound expressed as acetylsalicylic acid.

19. The pharmaceutical form of claim 16, in which the combination is prepared from a mixture in which at least 50% by weight of the first compound and at least 50% of the second compound are in soluble form.

20. The pharmaceutical form of claim 16, in which the combination is prepared from a mixture in which at least 90% by weight of the first compound and at least 90% of the second compound are in soluble form.

21. The pharmaceutical form of claim 16, in which the combination is prepared from a mixture in which the first compound and the second compound are substantially completely in soluble form.

22. The pharmaceutical form of claim 16, in which the combination is in the form of dry particles, especially micro particles, prepared by drying a mixture in which the first compound and the second compound are partly in a soluble form.

23. The pharmaceutical form of claim 16, in which the combination is in the form selected from the group consisting of a matrix, a gel, an hydrogel, a wax and a porous carrier and combinations thereof.

24. The pharmaceutical form of claim 16, which is at least a controlled release formulation for the second compound.

25. The pharmaceutical form of claim16, which is at least an immediate release formulation for the first compound.

26. The pharmaceutical form of claim 16, which further comprises at least one compound reacting in presence of water so as to prepare substantially immediately a solution or suspension of first compound and second compound.

27. The pharmaceutical form of claim 16, in which second compound is glycine betaine or a pharmaceutical salt thereof.

28. A kit for a daily administration, said kit comprising at least:

- An first oral formulation comprising a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, and pharmaceutical derivatives thereof, and
- A second oral formulation comprising a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, or a pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound

in which the first oral formulation comprises less than 100 mg of said first compound expressed as acetylsalicylic acid, and

in which the amount of second compound in the second oral formulation is at least three times the amount, calculated as acetylsalicylic acid, of said first compound.

29. The kit of claim 28, in which the first oral formulation comprises an amount of said first compound, calculated as acetylsalicylic acid, of less than 85 mg, advantageously of less than 75 mg, preferably of less than 60 mg.

30. The kit of claim 28, in which the first oral formulation comprises an amount of acetylsalicylic acid or pharmaceutical derivative thereof

corresponding to 3 to 80 mg, advantageously from 5 to 75 mg, preferably from 10 to 75 mg calculated as acetylsalicylic acid.

31. The kit of claim 28, in which the second oral formulation comprises an amount of second compound corresponding to at least 5 times the amount by weight, calculated as acetylsalicylic acid, of said first compound.

32. The kit of claim 28,n which the second oral formulation comprises an amount of second compound corresponding to 10 times to 100 times by weight, calculated as acetylsalicylic acid, of said first compound.

33. The kit of claim 28, in which the first oral formulation comprises an amount of a second compound selected from the group consisting of betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound.

34. The kit of claim 28, in which the first oral formulation is prepared at least from a mixture in which at least 50% by weight of the first compound and at least 50% of the second compound are in soluble form.

35. The kit of claim 28, in which the first oral formulation is prepared at least from a mixture in which at least 90% by weight of the first compound and at least 90% of the second compound are in soluble form.

36. The kit of claim 28, in which the first oral formulation is prepared at least from a mixture in which the first compound and the second compound are substantially completely in soluble form.

37. The kit of claim 28, n which the second oral compound is at least in a controlled release form.

38. The kit of claim 28, in which the first oral compound is at least in a immediate release form.

39. The kit of claim 28, in which the second oral formulation is at least glycine betaine and its pharmaceutically acceptable salts.

40. The use of

- a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and
- a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound,

for the preparation of a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical dosage form according to anyone of the claims 16 to 27 or a kit according to any one of the claims 28 to 39, for treating or preventing blood flow disturbances.

41. The use of

- a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and
- a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof,

precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound,

for the preparation of a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical dosage form according to anyone of the claims 16 to 27 or a kit according to any one of the claims 28 to 39, for treating or preventing cancer.

42. The use of

- a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and
- a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound,

for the preparation of a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical dosage form according to anyone of the claims 16 to 27 or a kit according to any one of the claims 28 to 39, for treating or preventing diabetes.

43. The use of

- a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and
- a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound,

for the preparation of a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical dosage form according to anyone of the claims 16 to 27 or a kit according to any one of the claims 28 to 39, for treating or preventing gut.

44. The use of

- a first compound selected among the group consisting acetylsalicylic acid, salicylic acid, pharmaceutical derivatives thereof, and
- a second compound selected from the group consisting of lipidic betaines, betaines lipids, betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof, with the provision that said second compound is different from the first compound,

for the preparation of a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical dosage form according to anyone of the claims 16 to 27 or a kit according to any one of the claims 28 to 39, for treating or preventing inflammation.

45. Process of treatment of a patient in need for treating, preventing, reducing thrombosis troubles for a patient, by administering to said patient a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical unit dose according to anyone of the claims 16 to 27, in which advantageously before and/or during and/or after said administration, a therapeutic effective amount of glycine betaine is further administered to said patient.

46. Process of treatment of a patient in need for treating, preventing, reducing inflammation troubles in a patient, by administering to said patient a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical unit dose according to anyone of the claims 16 to 27, in which

advantageously before and/or during and/or after said administration, a therapeutic effective amount of glycine betaine is further administered to said patient.

47. Process of treatment of a patient in need for treating, preventing, reducing inflammation troubles in a patient, by administering to said patient a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical unit dose according to anyone of the claims 16 to 27, in which advantageously before and/or during and/or after said administration, a therapeutic effective amount of glycine betaine is further administered to said patient.

48. Process of treatment of a patient in need for treating, preventing, reducing inflammation troubles in a patient, by administering to said patient a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical unit dose according to anyone of the claims 16 to 27, in which advantageously before and/or during and/or after said administration, a therapeutic effective amount of glycine betaine is further administered to said patient.

49. Process of treatment of a patient in need for treating, preventing, reducing gut troubles in a patient, by administering to said patient a pharmaceutical combination according to anyone of the claims 1 to 15 or a pharmaceutical unit dose according to anyone of the claims 16 to 27, in which advantageously before and/or during and/or after said administration, a therapeutic effective amount of glycine betaine is further administered to said patient.

50. A pharmaceutical composition comprising a betaine and aspirin in a formulation wherein the betaine and aspirin are formulated together in a

bilayered tablet, the aspirin being present in a first layer, and the betaine being present in a second layer in an amount at least three times the amount of aspirin.

51. The pharmaceutical composition as defined in claim 50, wherein the layer containing the betaine also includes one or more buffering agents.

52. The pharmaceutical composition as defined in claim 50, wherein the tablet includes a core and a coating layer surrounding said core and wherein one of the betaine and aspirin is present in the core and the other is present in a coating layer surrounding the core.

53. The pharmaceutical composition as defined in claim 50, wherein the tablet includes a core and a coating layer surrounding said core and wherein a mixture of the betaine and aspirin is present in the core and one of the betaine and aspirin is present in the coating layer surrounding the core.

54. The pharmaceutical composition as defined in claim 52, wherein the aspirin is present in the core and the betaine is present in the coating layer.

55. The pharmaceutical composition as defined in anyone of the claims 52 to 54, wherein the aspirin is present in the core and the betaine present in the coating layer is in a controlled release form.

56. The pharmaceutical composition as defined in anyone of the claims 52 to 54, wherein the betaine is present in the core in a controlled release form and the aspirin is present in the coating layer.

57. The pharmaceutical composition as defined in claim 53 wherein the coating layer also includes one or more buffering agents.

58. The pharmaceutical composition as defined in claim 54 wherein the coating layer also includes one or more buffering agents and one or more protecting films.

59. The pharmaceutical composition as defined in claim 50 wherein the betaine is selected from the group consisting of betaines of formula $(CH_3)_3N^+(CH_2)_nCOO^-$ with n an integer from 1 to 5, pharmaceutically acceptable salts thereof, esters thereof, precursors thereof, and mixtures thereof.

60. The pharmaceutical composition as defined in claim 50 further including an outer protective coating or finishing layer surrounding said tablet.

61. The pharmaceutical composition as defined in claim 50 wherein the aspirin is in the form of enteric coated aspirin granules.

62. The pharmaceutical composition as defined in claim 1 in the form of a bilayered tablet which comprises a first layer comprising aspirin granules and one or more excipients, and a second layer comprising a betaine and one or more buffering compounds and one or more excipients.

63. The pharmaceutical composition as defined in claim 60, wherein the first layer comprises aspirin granules, one or more bulking agents and optionally a lubricant, and the second layer comprises a betaine, optionally a wet granulating agent, one or more buffering compounds selected from the group consisting of calcium carbonate, magnesium oxide, magnesium carbonate and mixtures thereof, and optionally magnesium stearate.

64. The pharmaceutical compositions as defined in anyone of the claims 50 to 63 further including an outer protective coating surrounding said bilayered tablet.

65. The pharmaceutical compositions as defined in anyone of the claims 50 to 63 further including an antithrombotic agent.

66. The pharmaceutical composition as defined in anyone of the claims 50 to 63 further including an anti cancerous agent.

67. The pharmaceutical composition as defined in anyone of the claims 50 to 63 further including an anti inflammatory agent.

68. The pharmaceutical composition as defined in anyone of the claims 50 to 63 further including an antibiotic agent.

69. The pharmaceutical composition as defined in anyone of the claims 50 to 63 further including an anti diabetic agent.

70. The pharmaceutical composition as defined in anyone of the claims 50 to 63 further including an antioxidant agent

71. A method for preventing or inhibiting or treating atherosclerosis or reducing risk of or treating a cardiovascular event or disease, coronary artery disease or cerebro-vascular disease, which comprises administering to a patient in need of treatment a therapeutically effective amount of a pharmaceutical composition according to claim 50.

72. The method as defined in claim 71, wherein the betaine employed is anhydrous and/or monohydrate salt, and/or lipidic betaine and/or betaine lipids.

73. A pharmaceutical composition comprising betaine and aspirin in a formulation to reduce aspirin side effects wherein the betaine and aspirin are

formulated together in a bilayered tablet, the aspirin being present in a first layer, and the betaine being present in a second layer.

74. A pharmaceutical composition comprising betaine and aspirin in a formulation to increase aspirin therapeutic effects wherein the betaine and aspirin are formulated together in a bilayered tablet, the aspirin being present in a first layer, and the betaine being present in a second layer.

INTERNATIONAL SEARCH REPORT

International Application No
../IB 02/04923

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61K31/616 A61K31/205 A61P9/00 A61P1/00
A61P3/10 A61P7/02 A61P35/00 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 590 M (A. BEAUFOUR) 15 June 1964 (1964-06-15) cited in the application page 3, left-hand column, paragraph 4; claims; examples 1,2 pages 1-2 ----- -/--	1-3,11, 12,16, 22,23, 28-30, 33, 40-50, 65-74

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2004	02/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Kanbier, D

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GURFINKEL ET AL: "Fast Platelet suppressionby lycine acetylsalicylate in chronic stable coronary patients, potential clinical impact over regular aspirin for coronary syndromes" JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 35, no. 2Suppl.A, 2000, pages 408A-409A, XP008037129 the whole document	16,17, 40,45,71
X	ZOELLEI I ET AL: "BETAINE-PALMITATE REDUCES ACETYLSALICYLIC ACID-INDUCED GASTRIC DAMAGE IN RATS" SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY, XX, XX, vol. 36, no. 8, August 2001 (2001-08), pages 811-816, XP001027910 ISSN: 0036-5521 pages 812-815	16,27, 44, 46-48, 73,74
X	FR 2 403 799 A (UNION PHARMA SCIENT APPL) 20 April 1979 (1979-04-20) claims 1-3	16,17, 73,74
X	US 4 703 045 A (GUINOT PHILIPPE M) 27 October 1987 (1987-10-27) cited in the application	16-18, 40,43,49
A	column 6, lines 10-17; claim 15; examples 4,19	4,31,32
X	US 5 961 999 A (LANG GUENTHER ET AL) 5 October 1999 (1999-10-05) column 2, lines 32-34; example 3 column 1, lines 48-50	16-18,26
A	WO 99/45913 A (MERCK & CO INC ; NICHTBERGER STEVEN A (US)) 16 September 1999 (1999-09-16) page 29, lines 23-26 page 31, lines 11-23	1-74
A	US 4 066 756 A (ORR THOMAS SAMUEL CAMPBELL ET AL) 3 January 1978 (1978-01-03) column 1; claims 1,2,4,10,11; example 4	1-74
A	WO 02/066002 A (GLAXO WELLCOME SA ; IBANEZ MATILDE FERNANDEZ (ES); SANZ EMILIO GARRIZ) 29 August 2002 (2002-08-29) page 4, paragraph 3; claim 7	1-74
A	WO 00/51596 A (MESSADEK JALLAL) 8 September 2000 (2000-09-08) cited in the application the whole document	40,42, 43,45, 49,71

-/--

INTERNATIONAL SEARCH REPORT

International Application No
.../IB 02/04923

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BE 1 012 546 A (MESSADEK JALLAL) 5 December 2000 (2000-12-05) the whole document	40,42, 43,45, 49,71
A	BE 1 012 712 A (MESSADEK JALLAL) 6 February 2001 (2001-02-06) the whole document	40,42, 43,45, 49,71
A	WO 97/06795 A (KALVINSH IVARS ; VEVERIS MARIS (LV)) 27 February 1997 (1997-02-27) the whole document	40,42, 43,45, 49,71
A	WO 98/56497 A (RHODIA) 17 December 1998 (1998-12-17) page 17, lines 31-35; claims 42,43 page 11, lines 9-25 page 1, lines 5-7	13,16

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/IB 02/04923

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: -
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 45-49, 71, 72 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 45-49, 71, 72 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: -

Present claims 1, 16, 28 and 40-49 relate to compositions, uses and processes involving an extremely large number of possible compounds by use of the expressions "derivatives", "betaines lipids", "esters" and "precursors". Due thereto, a lack of clarity (and conciseness) within the meaning of Article 6 PCT arises to such an extent as to have rendered a meaningful search over the complete scope of the claims impossible.
Furthermore, the disease states as claimed in present use / process claims 40, 43, 44 and 46-49 are very general descriptions, which involve various editing errors. In claim 43, the disease state is defined as "gut"; in view of present claim 49, it would seem obvious that "gut troubles" is meant. Claims 46, 47 and 48 are identical; however, since it is not obvious here what subject matter is meant to be claimed, no other assumptions have been made for these claims.

Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely the lipidic and other betaines described on page 11 of the description, the aspirin and other salicyl compounds described on pages 11-12 and any compounds used in the examples; diseases states have been searched by taking into account the general idea of the application.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

ormation on patent family members

International Application No
../IB 02/04923

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
FR 2590	M		NONE		
FR 2403799	A	20-04-1979	FR	2403799 A2	20-04-1979
US 4703045	A	27-10-1987	BE	900660 A1	25-03-1985
			DE	3435040 A1	04-04-1985
			JP	1747136 C	25-03-1993
			JP	4032802 B	01-06-1992
			JP	60092215 A	23-05-1985
			US	4593020 A	03-06-1986
US 5961999	A	05-10-1999	DE	19520859 A1	12-12-1996
			BR	9602680 A	22-04-1998
			EP	0750904 A1	02-01-1997
			ES	2098207 T1	01-05-1997
			JP	8333217 A	17-12-1996
WO 9945913	A	16-09-1999	CA	2322824 A1	16-09-1999
			EP	1061908 A1	27-12-2000
			JP	2002506024 T	26-02-2002
			WO	9945913 A1	16-09-1999
			US	6136804 A	24-10-2000
			US	6511968 B1	28-01-2003
US 4066756	A	03-01-1978	GB	1509979 A	10-05-1978
WO 02066002	A	29-08-2002	WO	02066002 A2	29-08-2002
			EP	1363604 A2	26-11-2003
			JP	2004525897 T	26-08-2004
WO 0051596	A	08-09-2000	BE	1012495 A3	07-11-2000
			AU	2897900 A	21-09-2000
			WO	0051596 A1	08-09-2000
			BR	0008631 A	13-02-2002
			CA	2362558 A1	08-09-2000
			CN	1342071 T	27-03-2002
			EA	4047 B1	25-12-2003
			EP	1156796 A1	28-11-2001
			HU	0105397 A2	29-05-2002
			JP	2002538113 A	12-11-2002
			NZ	513905 A	28-09-2001
			PL	350408 A1	02-12-2002
			US	2004033223 A1	19-02-2004
			US	2002065320 A1	30-05-2002
BE 1012546	A	05-12-2000	BE	1012546 A6	05-12-2000
BE 1012712	A	06-02-2001	BE	1012712 A6	06-02-2001
WO 9706795	A	27-02-1997	LV	11727 A	20-04-1997
			LV	11727 B	20-08-1997
			WO	9706795 A1	27-02-1997
WO 9856497	A	17-12-1998	AT	246957 T	15-08-2003
			AU	753011 B2	03-10-2002
			AU	8066298 A	30-12-1998
			BR	9810023 A	15-01-2002
			CA	2297185 A1	17-12-1998

INTERNATIONAL SEARCH REPORT

ormation on patent family members

International Application No
/IB 02/04923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9856497 A		CA 2380311 A1	17-12-1998
		CN 1389538 A	08-01-2003
		CN 1263481 T	16-08-2000
		DE 69817182 D1	18-09-2003
		DE 69817182 T2	17-06-2004
		DK 993334 T3	08-12-2003
		EP 1323888 A1	02-07-2003
		EP 0993334 A1	19-04-2000
		ID 27732 A	26-04-2001
		NO 996089 A	08-02-2000
		NO 20030096 A	08-02-2000
		RU 2198906 C2	20-02-2003
		RU 2217585 C1	27-11-2003
		US 6258859 B1	10-07-2001
		WO 9856497 A1	17-12-1998
		US 2003040546 A1	27-02-2003
		US 6482866 B1	19-11-2002
		US 2004082484 A1	29-04-2004
		US 2004176478 A1	09-09-2004